


0625 Rec'd PCT/PTO 10 MAR 2000

FORM PTO-1390 (REV 12-29-99)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER	
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371				SPO-108	
				U.S. APPLICATION NO. (if known, see 37 CFR 1.5) <b>09/508342</b>	
INTERNATIONAL APPLICATION NO. PCT/JP98/04125		INTERNATIONAL FILING DATE September 11, 1998		PRIORITY DATE CLAIMED September 12, 1997	
TITLE OF INVENTION Mammalian Genes Involved in Circadian Periods					
APPLICANT(S) FOR DO/EO/US Yoshiyuki Sakaki, Hajime Tei					
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:					
<ol style="list-style-type: none"><li>1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</li><li>2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</li><li>3. <input checked="" type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).</li><li>4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.</li><li>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2))<ol style="list-style-type: none"><li>a. <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau).</li><li>b. <input checked="" type="checkbox"/> has been transmitted by the International Bureau.</li><li>c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</li></ol></li><li>6. <input checked="" type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)).</li><li>7. <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))<ol style="list-style-type: none"><li>a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau).</li><li>b. <input type="checkbox"/> have been transmitted by the International Bureau.</li><li>c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</li><li>d. <input type="checkbox"/> have not been made and will not be made.</li></ol></li><li>8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</li><li>9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). (unsigned)</li><li>10. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</li></ol>					
Items 11. to 16. below concern document(s) or information included:					
<ol style="list-style-type: none"><li>11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</li><li>12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</li><li>13. <input checked="" type="checkbox"/> A FIRST preliminary amendment. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.</li><li>14. <input type="checkbox"/> A substitute specification.</li><li>15. <input type="checkbox"/> A change of power of attorney and/or address letter.</li><li>16. <input checked="" type="checkbox"/> Other items or information: Verified Statement Claiming Small Entity Status</li></ol>					

U.S. APPLICATION NO. <u>09/50832</u>		INTERNATIONAL APPLICATION NO. PCT/JP98/04125		ATTORNEY'S DOCKET NUMBER SPO-108	
17. <input checked="" type="checkbox"/> The following fees are submitted: <b>BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):</b> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO ..... <b>\$970.00</b> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO. .... <b>\$840.00</b> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... <b>\$690.00</b> International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... <b>\$670.00</b> International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) ..... <b>\$96.00</b> <b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>				<b>CALCULATIONS</b> PTO USE ONLY	
Surcharge of <b>\$130.00</b> for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	13 - 20 =	0	X \$18.00	\$ 0.00	
Independent claims	11 - 3 =	8	X \$78.00	\$ 624.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$260.00	\$ 0.00	
<b>TOTAL OF ABOVE CALCULATIONS =</b>				<b>\$1,464.00</b>	
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				\$ -732.00	
<b>SUBTOTAL =</b>				<b>\$ 732.00</b>	
Processing fee of <b>\$130.00</b> for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$ 0.00	
<b>TOTAL NATIONAL FEE =</b>				<b>\$ 732.00</b>	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). <b>\$40.00</b> per property				\$ 0.00	
<b>TOTAL FEES ENCLOSED =</b>				<b>\$ 732.00</b>	
				Amount to be refunded:	\$
				charged:	\$
a. <input type="checkbox"/> A check in the amount of \$_____ to cover the above fees is enclosed.					
b. <input checked="" type="checkbox"/> Please charge my Deposit Account No. <u>19-0065</u> in the amount of \$ <u>732.00</u> to cover the above fees. A duplicate copy of this sheet is enclosed.					
c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>19-0065</u> . A duplicate copy of this sheet is enclosed.					
<b>NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.</b>					
SEND ALL CORRESPONDENCE TO Doran R. Pace Saliwanchik, Lloyd & Saliwanchik A Professional Association 2421 N.W. 41st Street, Suite A-1 Gainesville, FL 32606					
				 SIGNATURE:	
				Doran R. Pace NAME	
				<u>38,261</u> REGISTRATION NUMBER	



#3

Applicant or Patentee: Yoshiyuki Sakaki, Hajime Tei Attorney's  
 Serial or Patent No. \_\_\_\_\_ Docket No SPO-108  
 Filed or Issued: March 10, 2000  
 For: Mammalian Genes Involved in Circadian Periods

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY  
 STATUS (37 CFR 1.9 (f) and 1.27 (b)) – INDIVIDUAL

As below named individual, I hereby declare that I qualify as defined in 37 CFR 1.9 (c) for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, to the Patent and Trademark Office, with regard to the invention entitled Mammalian Genes Involved in Circadian Periods described in

☐ the specification filed herewith  
☒ PCT application Serial No PCT/JP98/04125, filed September 11, 1998  
☐ patent no \_\_\_\_\_, issued \_\_\_\_\_

I have not assigned, granted, conveyed or licensed and am under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who could not be classified as an independent inventor under 37 CFR 1.9 (c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9 (d) or a nonprofit organization under 37 CFR 1.9 (e)

Each person, concern, or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey or license any rights in the invention is listed below.

☒ no such person, concern, or organization  
☐ persons, concerns, organizations listed below\*

\*NOTE. Separate verified statements are required from each named person, concern or organization having rights to the invention averring their status as small entities. (37 CFR 1.27)

FULL NAME \_\_\_\_\_  
 ADDRESS \_\_\_\_\_  
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

FULL NAME \_\_\_\_\_  
 ADDRESS \_\_\_\_\_  
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

FULL NAME \_\_\_\_\_  
 ADDRESS \_\_\_\_\_  
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change of status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28 (b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

<u>Yoshiyuki Sakaki</u>	<u>Hajime Tei</u>	
NAME OF INDIVIDUAL	NAME OF INDIVIDUAL	NAME OF INDIVIDUAL
<u>Yoshiyuki Sakaki</u>	<u>Hajime Tei</u>	
Signature of Individual	Signature of Individual	Signature of Individual
<u>April 20, 2000</u>	<u>April 20, 2000</u>	
Date	Date	Date

10/PRTS

## SPECIFICATION

## MAMMALIAN GENES INVOLVED IN CIRCADIAN PERIODS

5 Technical Field

The present invention relates to mammalian genes whose expression changes with a circadian period.

Background Art

10 Many biochemical processes, physiological processes, and behavioral processes in various organisms ranging from microorganisms to vertebrates exhibit circadian rhythms (Edmunds, L. N. J., Cellular and Molecular Basis of Biological Clock, Springer-Verlag, New York, 1988). Several genes have been  
15 suggested to be involved in circadian rhythms.

For example, two mammalian circadian clock mutations have been confirmed thus far. They are Clock of the mouse (Vitaterna, M. H., et al., Science 264: 719-725, 1994) and tau of the hamster (Ralph, M. R. and Menaker, M., Science 241: 1225-1227, 1988). The Clock  
20 gene has recently been identified and is believed to encode a transcription factor in the circadian clock (Moor, R. Y. and Eichler, V. B., Brain Res. 42: 201-206: 1972; Stephan, F. K. and Zucker, I., Proc. Natl. Acad. Sci. USA 69: 1583-1586, 1972). On the other hand, the tau gene has not yet been cloned.

25 The period (per) gene has been isolated from *Drosophila* as a gene necessary for the expression of circadian rhythms for locomotive activities and eclosion behavior (Konopka, R. J. and Benzer, S., Proc. Natl. Acad. Sci. USA 68: 2112-2116, 1971). In the brain of the fly the oscillation of the levels of the per mRNA  
30 and of the PERIOD (dPER) protein are thought to determine the rhythms (Hardin, P. E., et al., Nature 343: 536-540, 1990; Zerr, D. M., et al., J. Neurosci. 10: 2749-2762, 1990). However, per homologues in other organisms than insects have not been identified.

35 Disclosure of the Invention

An object of the present invention is to provide novel

mammalian proteins and the genes thereof that are involved in the circadian period. More specifically, the object is to provide mammalian proteins and the genes thereof that are functionally equivalent to those of the *Drosophila* period (per) gene product.

5 To attain the above object, the present inventors focused on a region expected to play a functionally important role within the *Drosophila* gene known to be involved in the circadian rhythms, and performed a type of PCR, which had been developed on our own, using the primers designed based on the sequence of the region. As a result,  
10 we succeeded in isolating a human gene that corresponds to the above-mentioned *Drosophila* gene. We also succeeded in isolating a mouse gene that corresponds to the human gene by using the isolated human gene as a probe. Furthermore, we analyzed structures of the proteins encoded by the human and the mouse genes thus isolated and  
15 discovered that these proteins highly conserve the functional domains and the structural domains that have been identified in the *Drosophila* protein. In addition, analysis of the expression of the isolated mouse gene in the suprachiasmatic nucleus, which is the region responsible for functioning as a circadian pacemaker in the  
20 mammalian brain, revealed that the expression of the gene fluctuates with a circadian period.

Namely, the present invention relates to proteins and the genes thereof that are involved in the circadian periods of mammals, and more specifically to

- 25 (1) a protein derived from a mammal whose expression level in the suprachiasmatic nucleus (SCN) fluctuates with a circadian period,  
(2) a protein of (1) wherein the mammal is a human,  
(3) a protein of (1) wherein the mammal is a mouse,  
(4) a protein involved in the formation of circadian rhythm in  
30 the suprachiasmatic nucleus (SCN) comprising the amino acid sequence described in SEQ ID NO: 1 or said sequence in which one or more amino acids are substituted, deleted, or added,  
(5) a protein involved in the formation of circadian rhythm in  
the suprachiasmatic nucleus (SCN) comprising the amino acid  
35 sequence described in SEQ ID NO: 2 or said sequence in which one or more amino acids are substituted, deleted, or added,

- (6) a protein involved in the formation of circadian rhythm in the suprachiasmatic nucleus (SCN) encoded by the DNA having a sequence described in SEQ ID NO: 3 or by DNA that hybridizes with the DNA described in SEQ ID NO: 3,
- 5 (7) a protein involved in the formation of circadian rhythm in the suprachiasmatic nucleus (SCN) encoded by the DNA having a sequence described in SEQ ID NO: 4 or by DNA that hybridizes with the DNA described in SEQ ID NO: 4,
- (8) DNA encoding any of the proteins of (1) to (5),
- 10 (9) DNA having the sequence described in SEQ ID NO: 3 or DNA that hybridizes with the DNA having the sequence described in SEQ ID NO: 3, wherein the DNA encodes a protein involved in the formation of circadian rhythm in the suprachiasmatic nucleus (SCN),
- (10) DNA having the sequence described in SEQ ID NO: 4 or DNA that
- 15 hybridizes with the DNA having the sequence described in SEQ ID NO: 4, wherein the DNA encodes a protein involved in the formation of circadian rhythm in the suprachiasmatic nucleus (SCN),
- (11) a vector carrying any of the DNA of (8) to (10),
- (12) a transformant expressibly retaining any of the DNA of (8)
- 20 to (10), and
- (13) a method for producing any of the proteins of (1) to (7), the method comprising culturing the transformant of (12).

Herein, the "circadian periods" means the activity rhythms with a period of approximately 24 hours which are observed in a wide  
25 variety of behaviors such as endocrine secretions and body temperature, blood pressure, sleep-wakefulness, and others of an organism.

The expression of the protein of the present invention oscillates autonomously with a circadian period in the  
30 suprachiasmatic nucleus (SCN), which is a major circadian pacemaker of the mammalian brain (Moor, R. Y. and Eichler, V. B., Brain Res. 42: 201-206: 1972; Stephan, F. K. and Zucker, I., Proc. Natl. Acad. Sci. USA 69: 1583-1586, 1972). The amino acid sequences of the proteins derived from the human and the mouse included in the present  
35 invention are shown in SEQ ID NO: 1 and SEQ ID NO: 2, respectively. The amino acid sequences of these two mammalian proteins fairly

homologous with that of the *Drosophila* protein (the period gene product) (Citri, Y., et al., Nature 326: 42-47, 1987). The period gene is required for the expression of the circadian rhythms of locomotive activities and hatching behavior in *Drosophila* (Konopka, R. J. and Benzer, S., Proc. Natl. Acad. Sci. USA 68: 2112-2116, 1971). The oscillations of its mRNA and protein levels in the fly brain are thought to determine the rhythms (Hardin, P. E., et al., Nature 343: 536-540, 1990; Zerr, D. M., et al., J. Neurosci. 10: 2749-2762, 1990). These two proteins show highly homologous with the *Drosophila* protein in the PAS domains which have been suggested to be structurally and functionally important based on the genetic and biochemical studies (Baylies, M. K. et al., Nature 326: 390-392, 1987; Saez, L. and Young, M. W., Neuron 17: 911-920, 1996).

Recently King et al. have cloned the mammalian "Clock" gene, which encodes a bHLH-PAS-polyQ polypeptide (King, D. P., et al., Cell 89: 641-653, 1997; Antoch, M. P., et al., Cell 89: 655-667, 1997). The proteins of the present invention can form dimers with other molecules such as "CLOCK" by means of the PAS-PAS interaction in the circadian clock system.

The proteins of the present invention can be prepared as a recombinant protein utilizing the genetic recombinant technology, or as a natural protein. A recombinant protein can be prepared by culturing the cells transformed with DNA encoding the protein of the present invention as described later. A natural protein can be isolated, for example, from the somatic cell tissues, such as brain, pancreas, kidney, skeletal muscle, liver, lung, placenta, heart, spleen, and testis using an affinity column with an appropriate carrier bound to an antibody that is prepared using the above-mentioned recombinant protein of the present invention.

It is possible for a person skilled in the art to prepare a protein substantially identical to the protein described in SEQ ID NO: 1 or SEQ ID NO: 2 by making amino acid substitutions and other modifications to the protein described in SEQ ID NO: 1 using known methods. Mutations of amino acids in a protein may also occur spontaneously. Thus, the present invention includes modified proteins that result from the modification of amino acids of the

protein described in SEQ ID NO: 1 or 2 by substitution, deletion, or addition, and are involved in the formation of circadian rhythms in the suprachiasmatic nucleus (SCN). The known methods to modify amino acids include the ODA (Oligonucleotide-directed Dual  
5 Amber)-LA PCR method (Hashimoto-Gotoh, T., et al., Gene 152: 271-275, 1995). The amino acids to be substituted are usually within 10 amino acids, preferably within 6 amino acids, and more preferably within 3 amino acids.

It is routine for one skilled in the art to obtain proteins  
10 that are substantially functionally equivalent to the protein described in SEQ ID NO: 1 or 2 from DNAs that are highly homologous with the DNA having a sequence described in SEQ ID NO: 3 or 4 and isolated from other organisms using such methods as the known hybridization technique (Church, G. M. and Gilbert, W., Proc. Natl.  
15 Acad. Sci. USA 81: 1991-1995, 1984; Sambrook, J., et al., Molecular Cloning, 2<sup>nd</sup> ed., 1989) based on the DNA sequence described in SEQ ID NO: 3 or 4 (or part thereof). Thus the proteins encoded by the DNA that hybridizes with the DNA sequence described in SEQ ID NO: 3 or 4, which are involved in the formation of circadian rhythms  
20 in the suprachiasmatic nucleus (SCN), are also included in the proteins of the present invention. The source of the DNA for hybridization includes mammals such as rats, dogs, cats, monkeys, whales, cattle, pigs, and horses. The DNA encoding the proteins from these other organisms should usually highly homologous with  
25 the DNA described in SEQ ID NO: 3 or 4. "Being highly homologous" means having at least 60%, preferably at least 70%, more preferably at least 80%, and still more preferably at least 90% of sequence identity with the DNA described in SEQ ID NO: 3 or 4. The hybridization for isolating such DNAs can be performed, for example,  
30 in a mixture consisting of 6 x SSPE, 5 x Denhardt's solution, 0.5% SDS, 100 µl/ml denatured salmon sperm DNA, and 50% formamide, usually at 42°C, less stringently at 32°C, or more stringently at 65°C.

The present invention also relates to DNAs encoding the proteins of the present invention described above. The DNAs  
35 encoding the proteins of the present invention can be cDNA, genomic DNA, or synthetic DNA. The DNAs of the present invention can be



utilized, for example, to manufacture the proteins of the present invention as recombinant proteins. Namely, the DNA encoding a protein of the present invention (for example, the DNA described in SEQ ID NO: 3 or 4) is inserted into an appropriate expression  
5 vector, appropriate cells are transformed with the vector, the transformants are cultured, and the expressed protein is purified to prepare the proteins of the present invention as recombinant proteins.

The preferred cells used for the production of the recombinant  
10 proteins include E. coli, yeast, insect cells, and animal cells. The vectors used to express the recombinant proteins within these cells include the pET system, pAUR system, baculovirus vectors (pBlue Bac, etc.), and the CMV or RSV promoter-driven vectors, etc.

The transfection of the vector into the host cell can be done,  
15 for example, by electroporation for E. coli and yeast, and the liposome method for insect cells and animal cells. The lithium acetate method can also be used for yeast.

The recombinant protein can be purified from the transformant, for example, by ion exchange, gel filtration, or anti-Per antibody  
20 column chromatography.

The proteins or the DNAs of the present invention are applicable to treat disorders related to circadian rhythms, such as sleep phase delay syndrome, sleep phase progression syndrome, non-circadian sleep-wake syndrome, irregular sleep-wake disorder,  
25 and time difference syndrome (so-called jet lag). They are also applicable to the labor and health management of irregular night time workers and to prevention of night poriomania in dementia.

#### Brief Description of the Drawings

30 Figure 1 shows the amino acid sequences within the PAS repeats (arrows) that were used to design the primers for IMS-PCR.

Figure 2 is a photograph showing an electrophoresis image of 3 bp ladder markers that were electrophoresed on a 10% non-denaturing PAGE gel in a non-continuous buffer solution system. A 10 bp DNA  
35 ladder (BRL) was electrophoresed on lane M.

Figure 3 is a photograph showing an electrophoresis image of

the IMS-PCR product (lanes marked with arrows) that was electrophoresed along with 59 bp, 65 bp, and 68 bp of the 3 bp ladder markers (lanes marked with asterisks).

5 Figure 4 shows an amino acid sequence comparison among the PERIOD family members. hDIAL, mDIAL, and PERIOD indicate the human, the mouse, and the *Drosophila* version of PERIOD, respectively. Shaded or dotted boxes indicate homologous sequences, and C1 through C6 indicate regions conserved among different *Drosophila* species.

10 Figure 5 shows an amino acid sequence comparison among the PERIOD family members. hDIAL, mDIAL, and PERIOD indicate the human, the mouse, and the *Drosophila* version of PERIOD, respectively. Shaded or dotted portions indicate homologous sequences. Sequences corresponding to NLS, the PAS-A repeats, the PAS-B repeats, and CLD are underlined, and the TG repeats (the SG repeats in the human and  
15 mouse PER) are boxed. Amino acid identities between the human PERIOD and the mouse PERIOD are indicated by asterisks above the human PERIOD sequence. The identities and homologies between the mammalian PERIOD and the *Drosophila* PERIOD are indicated by asterisks and open circles below the *Drosophila* PERIOD sequence.

20 Figure 6 is a photograph showing the northern blot analysis of hPER. hPER was bound to the filter as a probe, and then G3PDH was bound as a loading control.

Figure 7 is a photograph showing the northern blot analysis of mPer. mPer was bound to the filter as a probe, and then G3PDH  
25 was bound as a loading control.

Figure 8 is a photograph showing the results of *in situ* hybridization of mPer in the mouse brain under the LD (top) and the DD (bottom) conditions. SCN is indicated by arrows. The bar indicates 2 mm.

30 Figure 9 shows the results of quantification of *in situ* hybridization data under the LD (top) and the DD (bottom) conditions. Each data point is the average  $\pm$  SEM (n=5). \*\* indicates significance at the 1% significance level, and \* at the 5% significance level, compared with the values at ZT16 and CT16. The  
35 white portion of the bar represents the light period, and the black portions the dark periods.

Figure 10 shows the results of the competitive RT-PCR analysis on the mPer mRNA under the LD (top) and the DD (bottom) conditions.  $\Delta$ mPer indicates a competitive factor for mPer and  $\Delta\beta$ -actin indicates a competitive factor for  $\beta$ -actin. The white portion of the bar represents the light period, and the black portions the dark periods.

#### Best Mode for Carrying out the Invention

The present invention is illustrated in detail below with reference to the following examples, but is not to be construed as being limited thereto.

#### Example 1 Isolation of the mammalian homologues of per

In order to isolate the mammalian homologues of per, the inventors have developed a novel method, intramodule scanning (IMS)-PCR. The principle of the method is based on the fact that in the human genome short stretches of DNA sequences (modules) that encode short polypeptide fragments (motifs) are scattered over long genomic distances. If a sufficient number of "intramodule scanning" primers are used to cover the entire length of a gene, the module can be screened with equal frequencies irrespective of their expression levels.

Genetic and biochemical studies have suggested that the PAS domains in dPER are structurally and functionally important (Baylies, M. K. et al., Nature 326: 390-392, 1987; Saez, L. and Young, M. W., Neuron 17: 911-920, 1996). Therefore, we designed 18 different primers corresponding to the internal sequences of the dPER PAS-A and PAS-B repeats (Figure 1). The sequences of the degenerate primer pairs for the PAS-A and PAS-B repeats are as follows:

GTGCTGGGCTACCCN(A/C)GNGA;  
30 CTGGGCTACCCCC(A/G)(A/G)GANATG;  
GGCTACCCCC(A/G)(A/G)GANATGTGG;  
CTGGGCT(A/T)CCTGCCNCA(A/G);  
CTGGGCT(A/T)CCTGCCNCA(A/G)GA;  
GGCTACCTGCC(C/T)CA(A/G)GAN(C/T);  
35 GCCCG(G/A)TCCTTCAG(G/A)TGNAC;  
TCCTCATG(A/G)TGCAC(A/G)(T/A)ANTC;

ATGTCCTCATG(A/G)TG(C/G)AC(A/G)(A/T)A; and  
GACAC(A/G)TCCTCATG(A/G)TG(A/G)TA.

Here, symbols such as A/G mean mixture primers between A and G.

Since homologous polypeptides share common characteristics  
5 at the corresponding positions within the molecules, when the  
corresponding amino acid sequences are used for synthesizing PCR  
primers, the lengths of the PCR products reflect the characteristics  
of the domain structure in each polypeptide with respect to the  
positions. Considering the lengths of a codon (3 bp) and an exon  
10 (100 bp on average) in a human gene, we synthesized the 3 bp ladder  
markers (53 to 113 bp) by PCR using the series of primers and pUC18  
as the template. An electrophoretic image of these 3 bp ladder  
marker and a 10 bp DNA ladder marker (BRL) are shown in Figure 2.  
The markers were electrophoresed along with the PCR products side  
15 by side in a non-continuous buffer solution system (Ito, T., Hohjoh,  
H. and Sakaki, Y., Electrophoresis 14: 278-282, 1993) on a non-  
denaturing PAGE (10%) gel (Figure 3).

Each PCR mixture (Sambrook, J., et al., Molecular Cloning,  
Cold Spring Harbor Laboratory, 1989) contained 0.5 µg of human  
20 genomic DNA. The mixture was incubated at 94°C for 1 minute, and  
subjected to 3 cycles of [94°C for 30 seconds, 37°C for 30 seconds,  
and 72°C for 30 seconds], followed by 25 cycles of [94°C for 30 seconds,  
45°C for 30 seconds, and 72°C for 30 seconds].

The DNA bands of expected lengths were cloned and their  
25 sequences determined. Among the 33 clones (59 to 74 bp) derived  
from the 12 bands that were produced by the nested PCR using a certain  
primer pair (corresponding to the peptide sequences 5'"GYLPQD" and  
3'"FVHHEDI"), the clones of 65 bp were especially amplified 6 to  
21 fold. It became clear that the genomic DNA sequence containing  
30 the 65 bp fragment has a 106 bp exon encoding 35 amino acid residues  
that are part of the PAS-B domain consisting of a total of 125 amino  
acids. We isolated the corresponding cDNA and named human PER (hPER)  
cDNA. Next, we cloned a mouse homologue (mPer) cDNA using the hPER  
cDNA as a probe. The nucleotide sequences determined are shown in  
35 SEQ ID NO: 3 for hPER, and SEQ ID NO: 4 for mPer. FISH revealed  
that the hPER gene and the mPer gene were located at 17p12-13.1 and

11B, respectively, which are gene loci in synteny between the two species.

The cDNA sequences of hPER and mPer contain ORF's that are expected to encode 1,290 amino acid residues and 1,291 amino acid residues, respectively. (See Figure 5. The putative amino acid sequence of the hPER gene product is shown in SEQ ID NO: 3, and that of the mPer gene product in SEQ ID NO: 4.) The amino acid identity between hPER and mPER is 92%, clearly indicating that hPER and mPer are conserved between the two species (Figure 5). A homology search using the BLAST program on non-overlapping amino acid databases demonstrated that the two mammalian PER's showed the highest homology with dPER (type A) (Citri, Y., et al., Nature 326:42-47, 1987). Significant homologies between the mammalian PER and the *Drosophila* PER were concentrated on five domains (Figures 4 and 5):

- I) N-terminal homologous regions (residues 44 to 131 of hPER and mPER);
- II) PAS-A (residues 217 to 282 for both homologues);
- III) PAS-B (residues 338 to 456 for both homologues) and its immediate downstream sequence (residues 457 to 485 for both homologues);
- IV) a short segment corresponding to the downstream region from the site (residue 589) of the per S mutation (which shortens the circadian period) (residues 624 to 645 for both homologues); and
- V) regions homologous with the PER-C C-terminal region (residues 1006 to 1050 for hPER and residues 1005 to 1049 for mPER), subsequent serine-glycine (SG) repeats (residues 1051 to 1072 for hPER and residues 1050 to 1071 for mPER), and further downstream homologous sequences (residues 1073 to 1108 for hPER and residues 1072 to 1107 for mPER).

The homology in these regions are 44%, 47%, 56%, 64%, and 37%, respectively (Figure 4). Although the PAS domains (regions II and III) of the PER homologues are fairly homologous to the corresponding region of dPER, other regions also show high homologies. Five structural domains and functional domains have been identified in dPER: a) the nuclear localization signal (NLS) (residues 66 to 79) (Vosshall, L. B., et al., Science 263: 1606-1609, 1996); b) the PAS domain (residues 233 to 490) necessary for dPER to interact with the NLS of TIM (Saez, L. and Young, M. W., Neuron 17: 911-920, 1996); c) the cytoplasmic localization domain (CLD)

(residues 453 to 511) located downstream from the PAS-B repeats (Saez, L. and Young, M. W., Neuron 17: 911-920, 1996); d) the PER-C domain (residues 524 to 685) which interacts with the PAS domain in the self-polypeptide (Huang, Z. J., et al., Science 267: 1169-1172, 1995); and e) the threonine-glycine (TG) repeats (residues 694 to 748) and the immediate downstream region (residues 749 to 868) which control the rhythm of the species-specific mating song of *Drosophila* (Wheeler, D. A., et al., Science 251: 1082-1085, 1991). Thus, NLS, PAS, CLD, the two domains within PER-C, and the TG repeats and a segment next to its C-terminus in each mammalian PER are arranged in exactly the same order as in dPER. Interestingly, the TG repeats of dPER are replaced with short SG repeats in the C-terminal halves of the PER homologues (Figure 5). This segment, which is adjacent to PER-C, and the sequence homologous to the C-terminal side of the TG repeats are located approximately 350 bases downstream from the original locations in dPER (Figure 4). These regions are also highly conserved in both the human and the mouse (Figure 5). Six PER segments (C1-C6) that are highly conserved among different *Drosophila* species are seen (Figure 4) (Colot, H. V., et al., EMBO J. 7: 3929-3937, 1988). Like in the silkworm homologue of PER, the parts of the mammalian PER that are homologous with dPER are concentrated on the regions corresponding to C1-C3 of dPER (Figure 4) (Reppert, S.M., et al., Neuron 13: 1167-1176, 1994). Considering these observations, hPER and mPer are conclusively the structural homologues of per.

#### Example 2 Expression of hPER and mPer

The expression patterns of hPER and mPer were examined by northern hybridization according to the method of Church and Gilbert (Church, G. M. and Gilbert, W., Proc. Natl. Acad. Sci. USA 81: 1991-1995, 1984). The filters were purchased from Clontech. The results are shown in Figure 6 (hPER) and Figure 7 (mPer). The expression product of approximately 4.6 kb was detected in all the tissues tested from the adult human and the mouse. However, the levels of the hPER/mPer transcription product are not uniform as compared with those of glycerol-3-phosphate dehydrogenase (G3PDH),

which is an enzyme in the glycolytic pathway and is abundantly and relatively constantly expressed in every cell. The wide distribution of the hPER/mPer expression is not surprising because in *Drosophila* the per expression has been detected in many tissues except the brain (Liu, X., et al., Genes Dev. 2: 228-238, 1988; Saez, L. and Young, M. W., Mol. Cell. Biol. 8: 5378-5385, 1988).

Example 3 Distribution of the mPer cDNA in the mouse brain

The distribution of the mPer cDNA in the mouse brain was examined by *in situ* hybridization. Continuous cortical sections (40  $\mu$ m thickness) of the mouse brain were prepared in the cryostat. *In situ* hybridization and determination of mRNA are described in the literature reference (e.g., Ban, Y., Shigeyoshi, Y. and Okamura, H., J. Neurosci. 17: 3920-3931, 1997). The  $^{32}$ P-labeled probes used in the hybridization were the sense and the antisense strands on the 5' side of the mPer cRNA (nucleotide positions 538-1752; data not shown). After the signals were converted into relative optical concentrations using the  $^{14}$ C-acrylic acid standard (Amersham, Inc. Plc.), the radioactivity was analyzed on each section on the BioMax film (Kodak) using a microcomputer connected to an image analyzer (MCID, Imaging Research, Inc.). These data were standardized against the difference in signal intensities between the equivalent regions of SCN and corpus callosum. The intensities of optical concentrations in the sections covering from the rostral end to the caudal end of SCN (10 pieces per mouse) were added, and the total was used as the measured value of the mPer mRNA quantity of this region. As a result, weak signals were detected from most brain areas including the cortical structures and non-cortical structures. Stronger mPer mRNA signals were detected from the pyramidal cell layer of piriform cortex, periventricular regions of the caudate putamen, many of the thalamic nuclei, and the granular layer of cerebellar cortex. Surprisingly, the highest mPer expression level in the brain was observed in SCN at a specific time (Figures 8 and 9; explained below).

In order to examine the time dependence of the mPer expression in SCN, mice were synchronized to an environment by keeping them

under the 12 h light/12 h dark (LD) conditions. The mPer mRNA was quantified by *in situ* hybridization and the competitive RT-PCR method. The competitive RT-PCR was performed as follows. First, we prepared mouse brain sections (0.5 mm thickness) in the "Mouse Brain Matrix" (Neuroscience, Inc., Tokyo). Using a microdissection needle (600  $\mu$ m diameter), SCN was pressed out laterally symmetrically from the frozen sections under a stereoscopic microscope. Total RNA was extracted from SCN (n=4) using TRIZOL solution (BRL), treated with DNase I (Stratagene), and purified using TRIZOL LS solution (BRL). "SUPERScript Preamplification System" (BRL) was used to reverse-transcribe approximately 1  $\mu$ g of RNA, and the cDNAs of mPer and  $\beta$ -actin were quantified by the competitive PCR method. The PCR products were electrophoresed on a non-denaturing PAGE gel (5.5%), stained with "SYBR Green" (Molecular Probes), and the DNA in appropriate bands was quantified with "FMBI011 fluoroimage analyzer" (Hitachi). The competitive DNA fragments for mPer and  $\beta$ -actin were constructed by making internal deletions in the respective cDNAs. mPer, mPer competitive factor,  $\beta$ -actin, and  $\beta$ -actin competitive factor were 482 bp, 246 bp, 1228 bp, and 1044 bp, respectively.

These two methods (*in situ* hybridization and the competitive RT-PCR method) produced similar oscillation profiles in LD (Figures 8 and 10; upper panels). The mPer mRNA quantity reached a peak in the light condition (from ZT4 to ZT8; ZT indicates the time under the LD condition as in Figures 8 to 10), and fell to a minimum in the dark condition (from ZT16 to ZT20) (Figure 9; upper panel). Moreover, under the constant dark condition (DD), there were free-run changes (Figures 8 and 10; lower panels), in which the mPer mRNA levels reached a peak between CT4 and CT8 (CT indicates the time under the DD condition as in Figures 8 to 10) and fell to a minimum between CT16 and CT20 (Figure 9; lower panel). The mPer mRNA in SCN is expressed with a strong and autonomous circadian period under the constant dark condition as described above, suggesting that this gene functions as a circadian rhythm pacemaker. Changes of the mPer mRNA in SCN with a circadian rhythm resemble the nervous activities in this brain region (Inouye, S-T. and



Kawamura, H., Proc. Natl. Acad. Sci. USA 76: 5962-5966, 1979;  
Schwartz, W. J. and Gainer, H., Science 197: 1089-1092, 1977;  
Gillette, M. U. and Reppert, S. M., Brain Res. Bull. 19: 135-139,  
1987), reaching a peak in the daytime and falling to a minimum during  
5 the night. mPer may function as a controlling factor of the nervous  
activities in SCN.

#### Industrial Applicability

10 The present invention provides novel mammalian proteins and  
their genes involved in the circadian period. The proteins and the  
DNAs of the present invention are expected to be able to correct  
abnormalities of the circadian rhythm in the mammals, and would thus  
be useful for treating disorders related to circadian rhythms, such  
as sleep phase delay syndrome, sleep phase progression syndrome,  
15 non-circadian sleep-wake syndrome, irregular sleep-wake disorder,  
and time difference syndrome (so-called jet lag). They are also  
applicable to the labor and health management of irregular night  
time workers and to the prevention of such disorders as night  
poriomania in dementia.

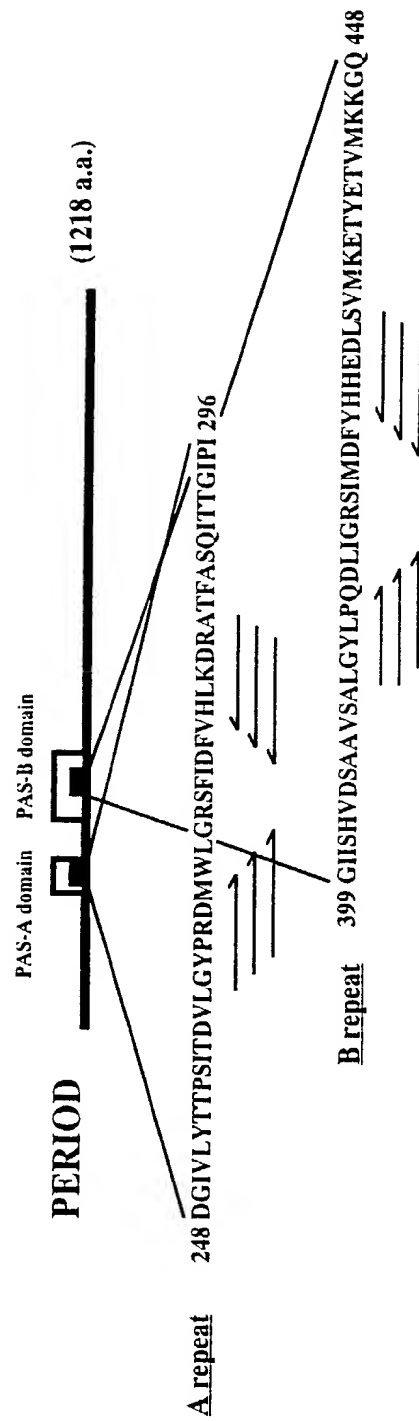
CLAIMS

1. A protein derived from a mammal whose expression level in the suprachiasmatic nucleus (SCN) fluctuates with a circadian period.
- 5 2. A protein of claim 1, wherein the mammal is a human.
3. A protein of claim 1, wherein the mammal is a mouse.
- ✓ 4. A protein involved in the formation of circadian rhythm in the suprachiasmatic nucleus (SCN) comprising the amino acid sequence described in SEQ ID NO: 1 or said sequence in which one or more amino  
10 acids are substituted, deleted, or added.
- ✓ 5. A protein involved in the formation of circadian rhythm in the suprachiasmatic nucleus (SCN) comprising the amino acid sequence described in SEQ ID NO: 2 or said sequence in which one or more amino acids are substituted, deleted, or added.
- 15 ✓ 6. A protein involved in the formation of circadian rhythm in the suprachiasmatic nucleus (SCN) encoded by the DNA having a sequence described in SEQ ID NO: 3 or by DNA that hybridizes with the DNA described in SEQ ID NO: 3.
- ✓ 7. A protein involved in the formation of circadian rhythm in  
20 the suprachiasmatic nucleus (SCN) encoded by the DNA having a sequence described in SEQ ID NO: 4 or by DNA that hybridizes with the DNA described in SEQ ID NO: 4.
8. DNA encoding the protein of any one of claims 1 to 5.
9. DNA having the sequence described in SEQ ID NO: 3 or DNA that  
25 hybridizes with the DNA having the sequence described in SEQ ID NO: 3, wherein the DNA encodes a protein involved in the formation of circadian rhythm in the suprachiasmatic nucleus (SCN).
10. DNA having the sequence described in SEQ ID NO: 4 or DNA that hybridizes with the DNA having the sequence described in SEQ ID NO:  
30 4, wherein the DNA encodes a protein involved in the formation of circadian rhythm in the suprachiasmatic nucleus (SCN).
11. A vector carrying the DNA of any one of claims 8 to 10.
12. A transformant expressibly retaining the DNA of any one of claims 8 to 10.
- 35 13. A method for producing the protein of any one of claims 1 to 7, the method comprising culturing the transformant of claim 12.

ABSTRACT

A human gene and a mouse gene corresponding to *Drosophila* period gene which is known to be involved in the circadian period.  
5 The proteins and DNAs are applicable to the treatment of diseases relating to the circadian rhythm such as sleep phase delay syndrom, sleep phase progression syndrom, non-circadian sleep-wake syndrome, irregular sleep-wake disorder, and time difference syndrome (so-called jet lag), and to the labor and health management of  
10 irregular night time workers and the prevention of such disorders as night poriomania in dementia.

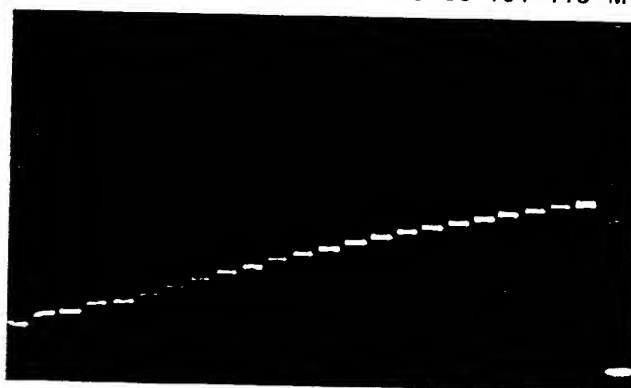
Figure 1



2/10

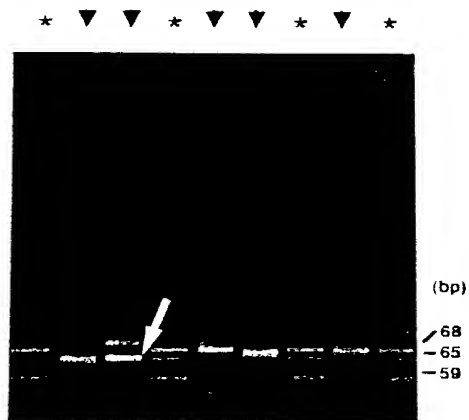
Figure 2

(bp) 53 57 60 65 71 77 83 89 95 101 107 113  
56 59 62 68 74 80 86 92 98 104 110 M



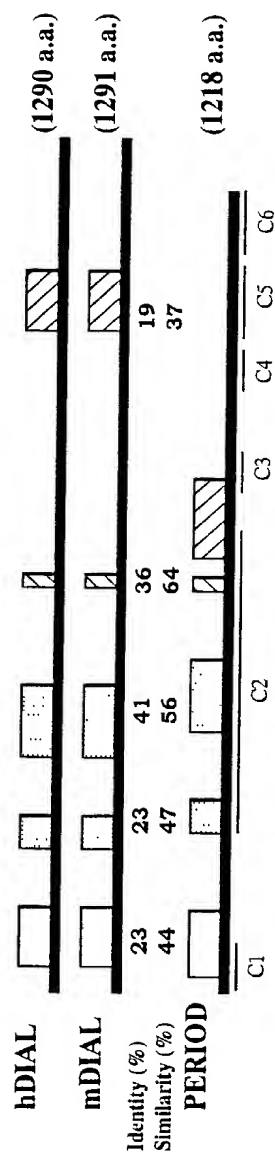
3/10

Figure 3



4/10

Figure 4



5/10

Figure 5

```

*****
hdIAL MSGPLEGADGGGDPFPGESPCPGVPSFGPPQHPCPGPSLADTDANSNGSSNGHESRGASQSSSSSSCNGKDSALLETTSSKSTNSQSPSPSSSIAYSL 110
mdIAL MSGPLEGADGGGDPFPGESPCPGVPSFGAPQHPCPGPSLADTDANSNGSSNGHESRGASQSSSSSSCNGKDSALLETTSSKSTNSQSPSPSSSIAYSL 110
PERIOD -----MEGGESTESTHNTKVSDSAYSNSCSNSQSQSGSSKSRISCSHSSGSSGIGGKPSIQASSSDMIKRNKDKSRGKKNKGAGGAGQAQTLI 92
*****

*****
hdIAL SASS-----EQDNFSTS GCSSEQSARARTQKELMTALRELKRLPERRGKG-RSGTLATLQYALACVKQVQANQYTTQQWS-LEEGE-PCSDMNSTITLELEHITS 210
mdIAL SASS-----EQDNFSTS GCSSEQSARARTQKELMTALRELKRLPERRGKG-RSGTLATLQYALACVKQVQANQYTTQQWS-LEEGE-PCSDMNSTITLELEHITS 210
PERIOD SASTSLGRDEENFRPSGTGCVQICRELQDQGHGHDSEPAIEQLQOEEDDSQSESEADRVGVAKS-EAAQFPPIF-SFLSVTVFPSSGCGGVGAAGLDSG 200
*****

*****
hdIAL EY-----TLNQDTPSYAVSELTNRVITSEDAVLLCKKROVERSTPSELAPQVGVFVCTSPSLTMT-GASAGSGLR 289
mdIAL EY-----TLNQDTPSYAVSELTNRVITSEDAVLLCKKROVERSTPSELAPQVGVFVCTSPSLTMT-GASAGSGLR 289
PERIOD LNKPKTWEAGPKLESMTGVGAAAGTCQGERVMDPFCVIEHDCIVLYTTPSIYVLYTROMYLCRSFIDFVHKDRAFA-SQITTPGIPASRVSVPKDAKS 309
*****

*****
hdIAL DFTQKSVFCRIR-CG-PDRDPCPRVQFRLT-PY--V-TKIRVSD-GAFAPQCCLL-X-ABRIHSGTEAP-RIPPEK-RIPTTHPTSCLPQVDRRAAPLGLTLPDL 388
mdIAL DFTQKSVFCRIR-CG-PDRDPCPRVQFRLT-PY--V-TKIRVSD-GAFAPQCCLL-X-ABRIHSGTEAP-RIPPEK-RIPTTHPTSCLPQVDRRAAPLGLTLPDL 388
PERIOD TFCVMLRRYRGLKSGGCGVIGRVPYSYEPRLGLTPREAPPEARPNIMVSNGTNMLLVICATTPIKSYKVPDETLGQSKPKFAIRHTATGIIISHVDSAAVSALGILPDL 419
*****

*****
hdIAL LGAPVILFLHPRPPLHAIHKRIQ-----LACOPFDSPIPCARNGEYVNDTSNAGFVHPMSKRVAPVLCRHKVRTAPLNEOVPTTFAFSPAPSLD-SQ-IQBLSEQI 493
mdIAL LGAPVILFLHPRPPLHAIHKRIQ-----LACOPFDSPIPCARNGEYVNDTSNAGFVHPMSKRVAPVLCRHKVRTAPLNEOVPTTFAFSPAPSLD-SQ-IQBLSEQI 493
PERIOD IGRSINDFYHEDLSVHKETITVTKRGCTAGASPCSKPIREPLIONGCIVLLETENISFVNPWSKRLKLVVGHHRVFGGKQCVPE-AAPT-C-KIKISBAQ---SRN- 523
*****

*****
hdIAL HRILLQPVHSSPTGLCGVAVTSPGLHSPGSSSDSNGGDAEGPGPAPVTPQOICKDVHLVKHQOQLFIESRARQSPRPLPATGTFKAKALCQSPDPELAGSP 603
mdIAL HRILLQPVHSSPTGLCGVAVTSPGLHSPGSSSDSNGGDAEGPGPAPVTPQOICKDVHLVKHQOQLFIESRARQSPRPLPATGTFKAKALCQSPDPELAGSP 603
PERIOD TRIKEDIVKRLAET---VSRP-SDTV-KQEVSRRCALASFM-ELMDEVSRADL-K-LEL-PHE-NELTVSRDSVMLGEISPHHDYDSKS-STETP----- 612
*****

*****
hdIAL VQAPLALVPERAEKKEASSC-----PQOIKTSLAYLESZLQDITKRRKASSSSYTTSSASDDDRQRTGPVSVGTIKDPFSAALS GEGATPRKEPVVGGTSLFIALANKAE 713
mdIAL DQASLALAPEPERKETSGC-----PQOIKTSLAYLESZLQDITKRRKASSSSYTTSSASDDDRQRTGPVSVGTIKDPFSAALS GEGATPRKEPVVGGTSLFIALANKAE 713
PERIOD -----PQOIKTSLAYLESZLQDITKRRKASSSSYTTSSASDDDRQRTGPVSVGTIKDPFSAALS GEGATPRKEPVVGGTSLFIALANKAE 553
*****

*****
hdIAL SVSVSTSCQSPSSSTIVHVGDKKPPESDIIMMEDLPLAGPAPSPAPSPVTPADPAPDATRVGLTKAVLSLHTQKRPQAPLSRPRDLGRLGLDSSSTAFSALGERGCH 823
mdIAL SVSVSTSCQSPSSSTIVHVGDKKPPESDIIMMEDLPLAGPAPSPAPSPVTPADPAPDATRVGLTKAVLSLHTQKRPQAPLSRPRDLGRLGLDSSSTAFSALGERGCH 820
PERIOD -----SVSVSTSCQSPSSSTIVHVGDKKPPESDIIMMEDLPLAGPAPSPAPSPVTPADPAPDATRVGLTKAVLSLHTQKRPQAPLSRPRDLGRLGLDSSSTAFSALGERGCH 820
*****

*****
hdIAL HGAPAPSPRRHHKRSKAKRSRHH---QNPRAEAPCTVSHSPVPVPSPTWPTTPATTFFPAVUQPYLPVFSRPGGPQPLPAPTSPVPPAFAFAPLVTPMVALVLPNLYLFTPT 931
mdIAL HGAPAPSPRRHHKRSKAKRSRHH---QNPRAEAPCTVSHSPVPVPSPTWPTTPATTFFPAVUQPYLPVFSRPGGPQPLPAPTSPVPPAFAFAPLVTPMVALVLPNLYLFTPT 930
PERIOD -----HGAPAPSPRRHHKRSKAKRSRHH---QNPRAEAPCTVSHSPVPVPSPTWPTTPATTFFPAVUQPYLPVFSRPGGPQPLPAPTSPVPPAFAFAPLVTPMVALVLPNLYLFTPT 930
*****

*****
hdIAL SSYPIGALQTPAEGPPTPASHSPSPSLPALPSPPHRPSPLFNSRCSPLQLNLLQLELFRAGAAGVAGGPGS-SQORPPSPAEKAPAEKAPAEKAPAEKAPAEKAPAEK 1040
mdIAL PSYPIGQAPVEGPTTASHSPSPSLPPFLSPPHRPSPLFNSRCSPLQLNLLQLELFRAGAAGVAGGPGS-SQORPPSPAEKAPAEKAPAEKAPAEKAPAEKAPAEK 1039
PERIOD -----SSYPIGALQTPAEGPPTPASHSPSPSLPALPSPPHRPSPLFNSRCSPLQLNLLQLELFRAGAAGVAGGPGS-SQORPPSPAEKAPAEKAPAEKAPAEKAPAEKAPAEK 689
*****

*****
hdIAL RLRLDLDEPRSTGSA-SQNRSGDGGSGC-----SGSHGQSTSA-SQNRSGDGGSGC-SQNRSGDGGSGC-SQNRSGDGGSGC-SQNRSGDGGSGC-SQNRSGDGGSGC 1119
mdIAL RLRLDLDEPRSTGSA-SQNRSGDGGSGC-----SGSHGQSTSA-SQNRSGDGGSGC-SQNRSGDGGSGC-SQNRSGDGGSGC-SQNRSGDGGSGC-SQNRSGDGGSGC 1118
PERIOD -----RLRLDLDEPRSTGSA-SQNRSGDGGSGC-SQNRSGDGGSGC-SQNRSGDGGSGC-SQNRSGDGGSGC-SQNRSGDGGSGC-SQNRSGDGGSGC 798
*****

*****
hdIAL QVTKYVLQDPILWLMANADQVMMTYQVPSRDMTSLVKQDRERLR-AMQKQPRFSEDQRELGAHVSWVRKQGLFRALDVMACVDCGSSSQDPGHFDDPLFSELDGLGL 1228
mdIAL QVTKYVLQDPILWLMANADQVMMTYQVPSRDMTSLVKQDRERLR-AMQKQPRFSEDQRELGAHVSWVRKQGLFRALDVMACVDCGSSSQDPGHFDDPLFSELDGLGL 1227
PERIOD ANDTLKMLEYSGPHGKRGSSH-SWEGANKPKQQLTLGTAIKGAAGSAGGAVGTGCVGSGGAGVAGGGSSTGVTAGTPEGRAITTSGTGTPGGAGGGGGGAGAAAAAG 907
*****

*****
hdIAL EPMEEGGGQSGSGGSGGEGGCEAAG--GAKASS-SQDLAMEEKEGRSSSSPALTAGNCTS 1290
mdIAL EPMEEGGGQSGSGGSGGEGGCEAAG--GAKASS-SQDLAMEEKEGRSSSSPALTAGNCTS 1291
PERIOD ASSSVGSSSTGCPSSYPTCTQNLNWPFFSVGITTPVHSTHTMAQSSFSAGLPPTTYIIPASLTPTSPTRSFRHKKHPKGGTDMPTTSQAAAAAAQAMP/LQIMAGVH 1017
*****

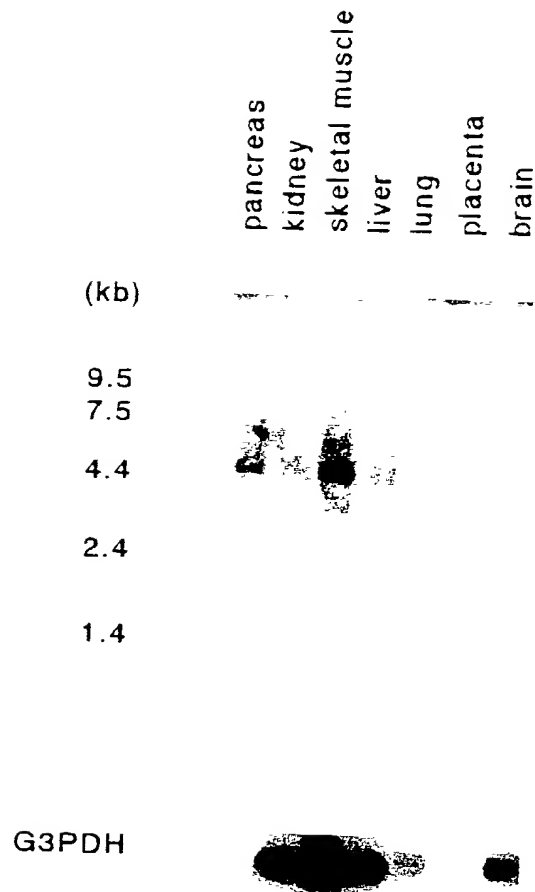
*****
PERIOD YPHPSLFTTHAAAAATAMHTQMPMPFGMANALQIPERPLGSQSAYNKSVYTTTTPASMTKKVPAGFHSVTTPAQVQRPSSQASVKTEPGFSAASVDPCKEVPDSSPIP 1127
PERIOD SVMGDYNSDPFCCSSNPNANKKYTDSNGNSDDMDGSSPSFYSSFIKTTDGSPPDEKDPKHKRLKSMSTSESKEHNPEDQTOHGDDG 1218

```



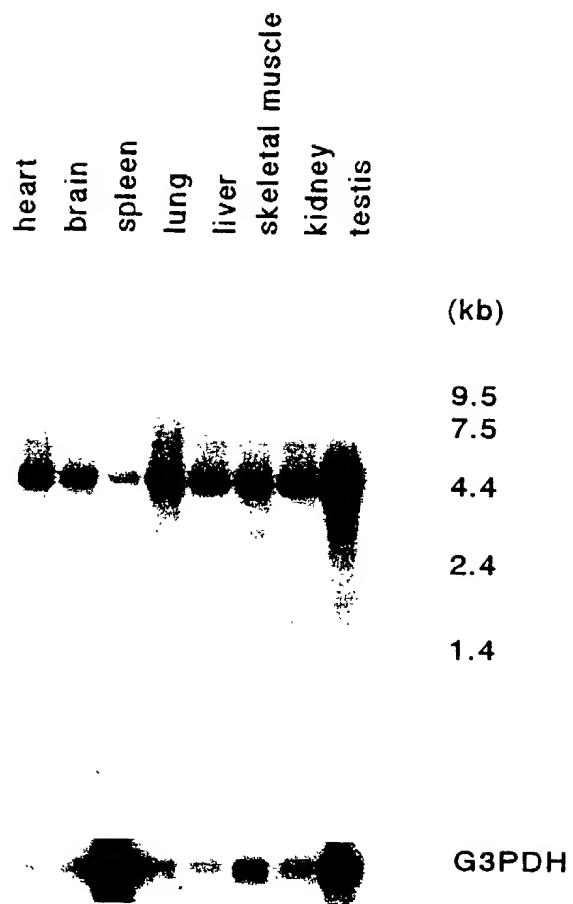
6/10

Figure 6



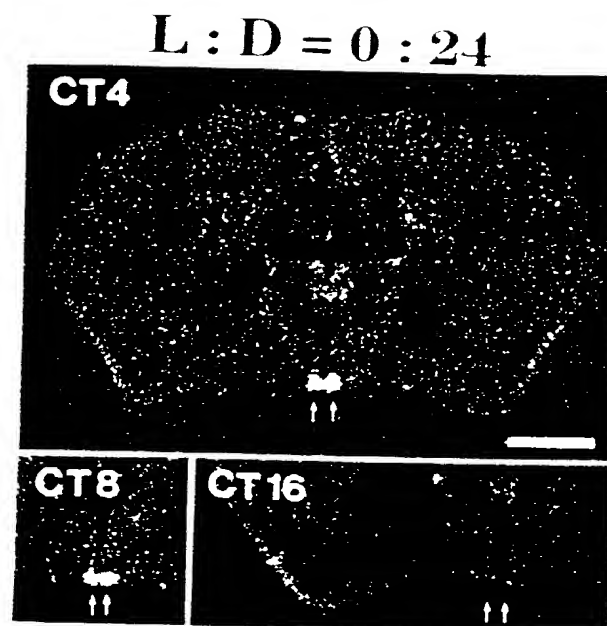
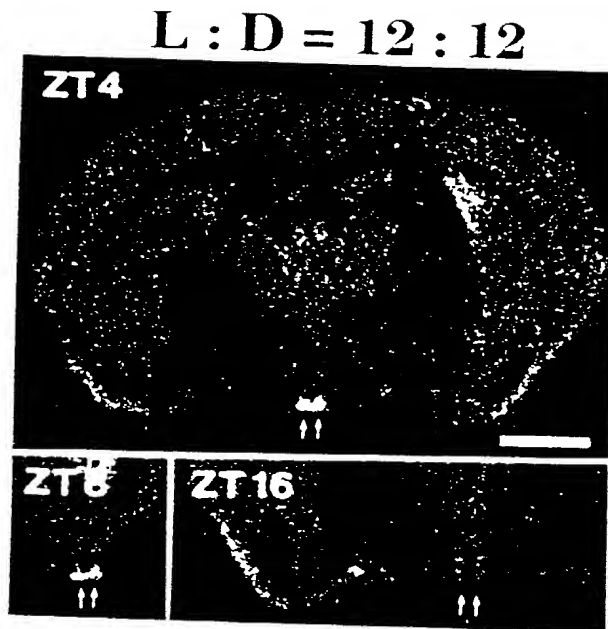
7/10

Figure 7



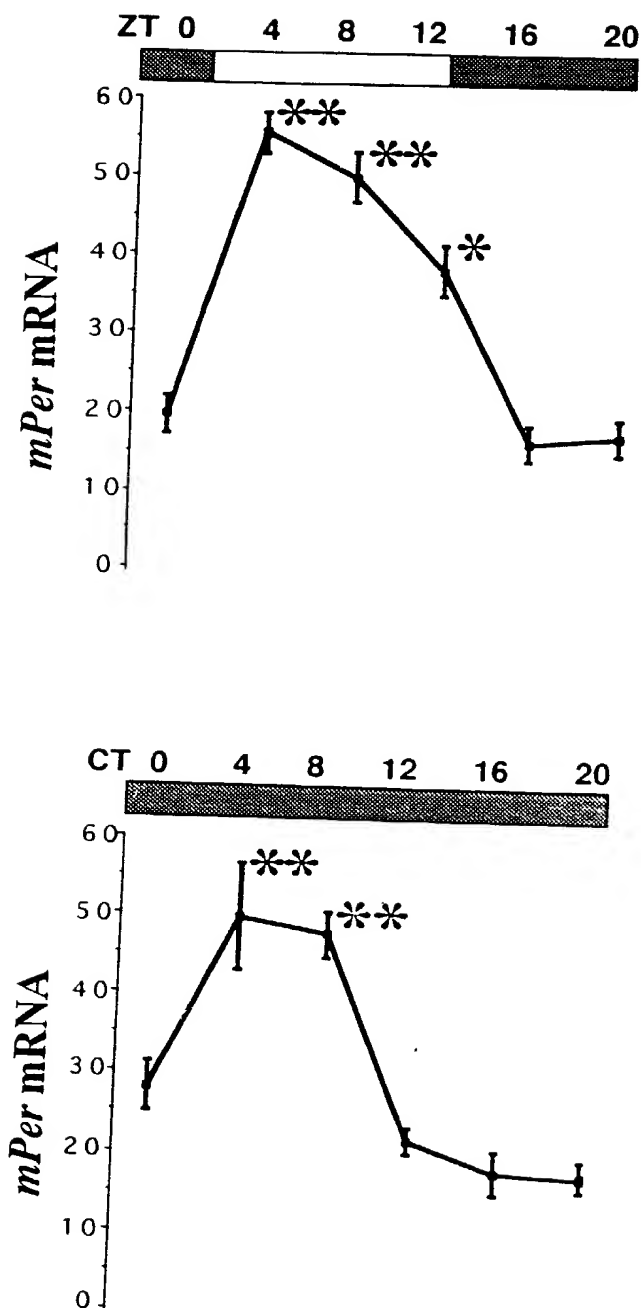
8/10

Figure 8



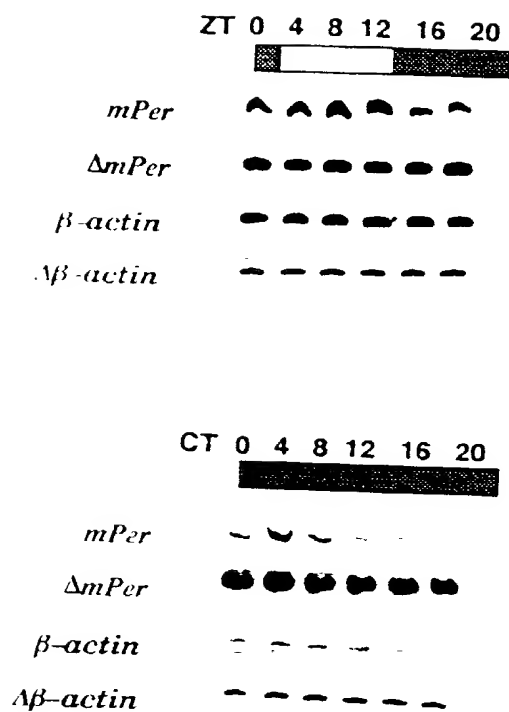
9/10

Figure 9



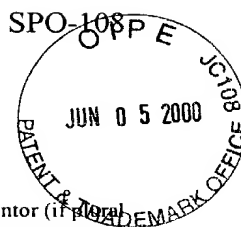
10/10

Figure 10



#4

Docket No. SPO-108



## DECLARATION (37 CFR 1.63) AND POWER OF ATTORNEY

As a below-named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name; and

I believe that I am the original, first, and sole inventor (if only one name is listed below), or an original, first, and joint inventor (if more than one name is listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **MAMMALIAN GENES INVOLVED IN CIRCADIAN PERIODS** the specification for which

☐ is attached hereto.

☒ was filed on September 11, 1998, as PCT International Application No. PCT/JP98/04125.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code §119 and/or §365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Application Serial No.	Country	Filing Date	Priority Claimed
9/267846	JP	September 12, 1997	Yes

I hereby claim priority benefits under Title 35, United States Code §119 of any provisional application(s) for patent listed below:

Application Serial No.	Filing Date	Priority Claimed
---------------------------	-------------	------------------

I hereby claim the benefit under Title 35, United States Code, §120 and/or §365 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status (patented, pending, abandoned)
---------------------------	-------------	--

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following persons registered to practice before the Patent and Trademark Office as my attorneys with full power of substitution and revocation to prosecute this application and all divisions and continuations thereof and to transact all business in the Patent and Trademark Office connected therewith: David R. Saliwanchik, Reg. No. 31,794; Jeff Lloyd, Reg. No. 35,589; Doran R. Pace, Reg. No. 38,261; Christine Q. McLeod, Reg. No. 36,213; Jay M. Sanders, Reg. No. 39,355; James S. Parker, Reg. No. 40,119; Jean Kyle, Reg. No. 36,987; Frank C. Eisenschenk, Reg. No. P-45,332; Seth M. Blum, Reg. No. P-45,489.

I request that all correspondence be sent to:

Doran R. Pace  
Saliwanchik, Lloyd & Saliwanchik  
2421 N.W. 41st Street, Suite A-1  
Gainesville, FL 32606-6669

I further request that all telephone communications be directed to:

Doran R. Pace  
352-375-8100

1-00  
Name of First or Sole Inventor Yoshiyuki SakakiResidence Kanagawa, Japan JPX Citizenship JapanesePost Office Address 2-51-42, Kamariyaminami, Kanazawa-ku, Yokohama-shiKanagawa 236-0045, JapanYoshiyuki SakakiDate April 20, 2000

Signature of First or Sole Inventor

\*\*\*\*\*

2-00  
Name of Second Joint Inventor Hajime TeiResidence Tokyo, Japan JPX Citizenship JapanesePost Office Address 1-5-10-503, Yayoi, Bunkyo-kuTokyo 113-0032, JapanHajime TeiDate April 20, 2000

Signature of Second Joint Inventor

\*\*\*\*\*

Name of Third Joint Inventor \_\_\_\_\_

Residence \_\_\_\_\_ Citizenship \_\_\_\_\_

Post Office Address \_\_\_\_\_

Date \_\_\_\_\_

Signature of Third Joint Inventor

\*\*\*\*\*

Name of Fourth Joint Inventor \_\_\_\_\_

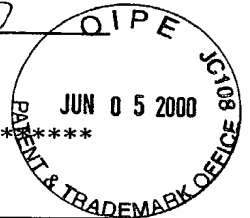
Residence \_\_\_\_\_ Citizenship \_\_\_\_\_

Post Office Address \_\_\_\_\_

Date \_\_\_\_\_

Signature of Fourth Joint Inventor

\*\*\*\*\*



## SEQUENCE LISTING

&lt;110&gt; SAKAKI, Yoshiyuki

&lt;120&gt; Mammalian Genes Involved in Circadian Periods

&lt;130&gt; SEN-903PCT

&lt;150&gt; JP 9-267846

&lt;151&gt; 1997-09-12

&lt;160&gt; 4

&lt;210&gt; 1

&lt;211&gt; 1290

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 1

Met Ser Gly Pro Leu Glu Gly Ala Asp Gly Gly Gly Asp Pro Arg Pro  
1 5 10 15

Gly Glu Ser Phe Cys Pro Gly Gly Val Pro Ser Pro Gly Pro Pro Gln  
20 25 30

His Arg Pro Cys Pro Gly Pro Ser Leu Ala Asp Asp Thr Asp Ala Asn  
35 40 45

Ser Asn Gly Ser Ser Gly Asn Glu Ser Asn Gly His Glu Ser Arg Gly  
50 55 60

Ala Ser Gln Arg Ser Ser His Ser Ser Ser Ser Gly Asn Gly Lys Asp  
65 70 75 80

Ser Ala Leu Leu Glu Thr Thr Glu Ser Ser Lys Ser Thr Asn Ser Gln  
85 90 95

Ser Pro Ser Pro Pro Ser Ser Ser Ile Ala Tyr Ser Leu Leu Ser Ala



100	105	110
Ser Ser Glu Gln Asp Asn Pro Ser Thr Ser Gly Cys Ser Ser Glu Gln		
115	120	125
Ser Ala Arg Ala Arg Thr Gln Lys Glu Leu Met Thr Ala Leu Arg Glu		
130	135	140
Leu Lys Leu Arg Leu Pro Pro Glu Arg Arg Gly Lys Gly Arg Ser Gly		
145	150	155 160
Thr Leu Ala Thr Leu Gln Tyr Ala Leu Ala Cys Val Lys Gln Val Gln		
	165	170 175
Ala Asn Gln Glu Tyr Tyr Gln Gln Trp Ser Leu Glu Glu Gly Glu Pro		
180	185	190
Cys Ser Met Asp Met Ser Thr Tyr Thr Leu Glu Glu Leu Glu His Ile		
195	200	205
Thr Ser Glu Tyr Thr Leu Gln Asn Gln Asp Thr Phe Ser Val Ala Val		
210	215	220
Ser Phe Leu Thr Gly Arg Ile Val Tyr Ile Ser Glu Gln Ala Ala Val		
225	230	235 240
Leu Leu Arg Cys Lys Arg Asp Val Phe Arg Gly Thr Arg Phe Ser Glu		
	245	250 255
Leu Leu Ala Pro Gln Asp Val Gly Val Phe Tyr Gly Ser Thr Ala Pro		
260	265	270
Ser Arg Leu Pro Thr Trp Gly Thr Gly Ala Ser Ala Gly Ser Gly Leu		
275	280	285
Arg Asp Phe Thr Gln Glu Lys Ser Val Phe Cys Arg Ile Arg Gly Gly		
290	295	300

Leu Leu Gln Pro Val His Ser Pro Ser Pro Thr Gly Leu Cys Gly Val  
500 505 510

Gly Ala Val Thr Ser Pro Gly Pro Leu His Ser Pro Gly Ser Ser Ser  
515 520 525

Asp Ser Asn Gly Gly Asp Ala Glu Gly Pro Gly Pro Pro Ala Pro Val  
530 535 540

Thr Phe Gln Gln Ile Cys Lys Asp Val His Leu Val Lys His Gln Gly  
545 550 555 560

Gln Gln Leu Phe Ile Glu Ser Arg Ala Arg Pro Gln Ser Arg Pro Arg  
565 570 575

Leu Pro Ala Thr Gly Thr Phe Lys Ala Lys Ala Leu Pro Cys Gln Ser  
580 585 590

Pro Asp Pro Glu Leu Glu Ala Gly Ser Ala Pro Val Gln Ala Pro Leu  
595 600 605

Ala Leu Val Pro Glu Glu Ala Glu Arg Lys Glu Ala Ser Ser Cys Ser  
610 615 620

Tyr Gln Gln Ile Asn Cys Leu Asp Ser Ile Leu Arg Tyr Leu Glu Ser  
625 630 635 640

Cys Asn Leu Pro Ser Thr Thr Lys Arg Lys Cys Ala Ser Ser Ser Ser  
645 650 655

Tyr Thr Thr Ser Ser Ala Ser Asp Asp Asp Arg Gln Arg Thr Gly Pro  
660 665 670

Val Ser Val Gly Thr Lys Lys Asp Pro Pro Ser Ala Ala Leu Ser Gly  
675 680 685

Glu Gly Ala Thr Pro Arg Lys Glu Pro Val Val Gly Gly Thr Leu Ser  
690 695 700

Pro Leu Ala Leu Ala Asn Lys Ala Glu Ser Val Val Ser Val Thr Ser

705	710	715	720
Gln Cys Ser Phe Ser Ser Thr Ile Val His Val Gly Asp Lys Lys Pro			
725	730	735	
Pro Glu Ser Asp Ile Ile Met Met Glu Asp Leu Pro Gly Leu Ala Pro			
740	745	750	
Gly Pro Ala Pro Ser Pro Ala Pro Ser Pro Thr Val Ala Pro Asp Pro			
755	760	765	
Ala Pro Asp Ala Tyr Arg Pro Val Gly Leu Thr Lys Ala Val Leu Ser			
770	775	780	
Leu His Thr Gln Lys Glu Glu Gln Ala Phe Leu Ser Arg Phe Arg Asp			
785	790	795	800
Leu Gly Arg Leu Arg Gly Leu Asp Ser Ser Ser Thr Ala Pro Ser Ala			
805	810	815	
Leu Gly Glu Arg Gly Cys His His Gly Pro Ala Pro Pro Ser Arg Arg			
820	825	830	
His His Cys Arg Ser Lys Ala Lys Arg Ser Arg His His Gln Asn Pro			
835	840	845	
Arg Ala Glu Ala Pro Cys Tyr Val Ser His Pro Ser Pro Val Pro Pro			
850	855	860	
Ser Thr Pro Trp Pro Thr Pro Pro Ala Thr Thr Pro Phe Pro Ala Val			
865	870	875	880
Val Gln Pro Tyr Pro Leu Pro Val Phe Ser Pro Arg Gly Gly Pro Gln			
885	890	895	
Pro Leu Pro Pro Ala Pro Thr Ser Val Pro Pro Ala Ala Phe Pro Ala			
900	905	910	

005000 "EHEP" 005000

[illegible]

Glu Ala Glu Ala Gly Ala Ala Arg Gly Gly Ala Glu Pro Gly Asp Gln  
1105                      1110                      1115                      1120



Val Ile Lys Tyr Val Leu Gln Asp Pro Ile Trp Leu Leu Met Ala Asn  
 1125 1130 1135

Ala Asp Gln Arg Val Met Met Thr Tyr Gln Val Pro Ser Arg Asp Met  
 1140 1145 1150

Thr Ser Val Leu Lys Gln Asp Arg Glu Arg Leu Arg Ala Met Gln Lys  
 1155 1160 1165

Gln Gln Pro Arg Phe Ser Glu Asp Gln Arg Arg Glu Leu Gly Ala Val  
 1170 1175 1180

His Ser Trp Val Arg Lys Gly Gln Leu Pro Arg Ala Leu Asp Val Met  
 1185 1190 1195 1200

Ala Cys Val Asp Cys Gly Ser Ser Thr Gln Asp Pro Gly His Pro Asp  
 1205 1210 1215

Asp Pro Leu Phe Ser Glu Leu Asp Gly Leu Gly Leu Glu Pro Met Glu  
 1220 1225 1230

Glu Gly Gly Gly Glu Gln Gly Ser Ser Gly Gly Gly Ser Gly Glu Gly  
 1235 1240 1245

Glu Gly Cys Glu Glu Ala Gln Gly Gly Ala Lys Ala Ser Ser Ser Gln  
 1250 1255 1260

Asp Leu Ala Met Glu Glu Glu Glu Gly Arg Ser Ser Ser Ser Pro  
 1265 1270 1275 1280

Ala Leu Pro Thr Ala Gly Asn Cys Thr Ser  
 1285 1290

<210> 2

<211> 1291

<212> PRT

<213> Mus musculus

Variable	Mean	Standard deviation	Minimum	Maximum
Age	34.2	10.5	18	65
Gender	0.45	0.50	0	1
Marital status	0.65	0.48	0	1
Education	12.5	2.1	9	16
Income	15.2	3.8	10	25
Health	0.75	0.42	0	1
Smoking	0.35	0.48	0	1
Alcohol	0.25	0.43	0	1
Exercise	0.40	0.50	0	1
Stress	0.60	0.49	0	1
Sleep	0.55	0.50	0	1
Appetite	0.65	0.48	0	1
Mood	0.70	0.46	0	1
Energy	0.60	0.49	0	1
Concentration	0.55	0.50	0	1
Memory	0.50	0.50	0	1
Emotion	0.65	0.48	0	1
Behavior	0.70	0.46	0	1
Thought	0.60	0.49	0	1
Feeling	0.75	0.42	0	1
Perception	0.65	0.48	0	1
Attention	0.55	0.50	0	1
Intuition	0.40	0.50	0	1
Imagination	0.30	0.45	0	1
Reasoning	0.50	0.50	0	1
Logic	0.45	0.50	0	1
Analysis	0.55	0.50	0	1
Synthesis	0.40	0.50	0	1
Evaluation	0.35	0.48	0	1
Problem solving	0.45	0.50	0	1
Decision making	0.50	0.50	0	1
Planning	0.40	0.50	0	1
Organization	0.35	0.48	0	1
Management	0.45	0.50	0	1
Leadership	0.30	0.45	0	1
Communication	0.55	0.50	0	1
Interpersonal skills	0.40	0.50	0	1
Teamwork	0.35	0.48	0	1
Conflict resolution	0.45	0.50	0	1
Networking	0.30	0.45	0	1
Public speaking	0.25	0.43	0	1
Writing	0.40	0.50	0	1
Reading	0.35	0.48	0	1
Listening	0.45	0.50	0	1
Observation	0.30	0.45	0	1
Imagination	0.25	0.43	0	1
Reasoning	0.40	0.50	0	1
Logic	0.35	0.48	0	1
Analysis	0.45	0.50	0	1
Synthesis	0.30	0.45	0	1
Evaluation	0.25	0.43	0	1
Problem solving	0.40	0.50	0	1
Decision making	0.35	0.48	0	1
Planning	0.45	0.50	0	1
Organization	0.30	0.45	0	1
Management	0.25	0.43	0	1
Leadership	0.40	0.50	0	1
Communication	0.35	0.48	0	1
Interpersonal skills	0.45	0.50	0	1
Teamwork	0.30	0.45	0	1
Conflict resolution	0.25	0.43	0	1
Networking	0.40	0.50	0	1
Public speaking	0.35	0.48	0	1
Writing	0.45	0.50	0	1
Reading	0.30	0.45	0	1
Listening	0.25	0.43	0	1
Observation	0.40	0.50	0	1
Imagination	0.35	0.48	0	1
Reasoning	0.45	0.50	0	1
Logic	0.30	0.45	0	1
Analysis	0.25	0.43	0	1
Synthesis	0.40	0.50	0	1
Evaluation	0.35	0.48	0	1
Problem solving	0.45	0.50	0	1
Decision making	0.30	0.45	0	1
Planning	0.25	0.43	0	1
Organization	0.40	0.50	0	1
Management	0.35	0.48	0	1
Leadership	0.45	0.50	0	1
Communication	0.30	0.45	0	1
Interpersonal skills	0.25	0.43	0	1
Teamwork	0.40	0.50	0	1
Conflict resolution	0.35	0.48	0	1
Networking</				

Ala Asn Gln Glu Tyr Tyr Gln Gln Trp Ser Leu Glu Glu Gly Glu Pro  
180 185 190

9/31

SPO-108

Cys Ala Met Asp Met Ser Thr Tyr Thr Leu Glu Glu Leu Glu His Ile  
195 200 205

Thr Ser Glu Tyr Thr Leu Arg Asn Gln Asp Thr Phe Ser Val Ala Val  
210 215 220

Ser Phe Leu Thr Gly Arg Ile Val Tyr Ile Ser Glu Gln Ala Gly Val  
225 230 235 240

Leu Leu Arg Cys Lys Arg Asp Val Phe Arg Gly Ala Arg Phe Ser Glu  
245 250 255

Leu Leu Ala Pro Gln Asp Val Gly Val Phe Tyr Gly Ser Thr Thr Pro  
260 265 270

Ser Arg Leu Pro Thr Trp Gly Thr Gly Thr Ser Ala Gly Ser Gly Leu  
275 280 285

Lys Asp Phe Thr Gln Glu Lys Ser Val Phe Cys Arg Ile Arg Gly Gly  
290 295 300

Pro Asp Arg Asp Pro Gly Pro Arg Tyr Gln Pro Phe Arg Leu Thr Pro  
305 310 315 320

Tyr Val Thr Lys Ile Arg Val Ser Asp Gly Ala Pro Ala Gln Pro Cys  
325 330 335

Cys Leu Leu Ile Ala Glu Arg Ile His Ser Gly Tyr Glu Ala Pro Arg  
340 345 350

Ile Pro Pro Asp Lys Arg Ile Phe Thr Thr Arg His Thr Pro Ser Cys  
355 360 365

Leu Phe Gln Asp Val Asp Glu Arg Ala Ala Pro Leu Leu Gly Tyr Leu  
370 375 380

Pro Gln Asp Leu Leu Gly Ala Pro Val Leu Leu Phe Leu His Pro Glu  
385 390 395 400





595					600					605						
Ala	Leu	Ala	Pro	Glu	Glu	Pro	Glu	Arg	Lys	Glu	Thr	Ser	Gly	Cys	Ser	
610					615					620						
Tyr	Gln	Gln	Ile	Asn	Cys	Leu	Asp	Ser	Ile	Leu	Arg	Tyr	Leu	Glu	Ser	
625					630					635					640	
Cys	Asn	Ile	Pro	Ser	Thr	Thr	Lys	Arg	Lys	Cys	Ala	Ser	Ser	Ser	Ser	
645					650					655						
Tyr	Thr	Ala	Ser	Ser	Ala	Ser	Asp	Asp	Asp	Lys	Gln	Arg	Ala	Gly	Pro	
660					665					670						
Val	Pro	Val	Gly	Ala	Lys	Lys	Asp	Pro	Ser	Ser	Ala	Met	Leu	Ser	Gly	
675					680					685						
Glu	Gly	Ala	Thr	Pro	Arg	Lys	Glu	Pro	Val	Val	Gly	Gly	Thr	Leu	Ser	
690					695					700						
Pro	Leu	Ala	Leu	Ala	Asn	Lys	Ala	Glu	Ser	Val	Val	Ser	Val	Thr	Ser	
705					710					715					720	
Gln	Cys	Ser	Phe	Ser	Ser	Thr	Ile	Val	His	Val	Gly	Asp	Lys	Lys	Pro	
725					730					735						
Pro	Glu	Ser	Asp	Ile	Ile	Met	Met	Glu	Asp	Leu	Pro	Gly	Leu	Ala	Pro	
740					745					750						
Gly	Pro	Ala	Pro	Ser	Pro	Ala	Pro	Ser	Pro	Thr	Val	Ala	Pro	Asp	Pro	
755					760					765						
Thr	Pro	Asp	Ala	Tyr	Arg	Pro	Val	Gly	Leu	Thr	Lys	Ala	Val	Leu	Ser	
770					775					780						
Leu	His	Thr	Gln	Lys	Glu	Glu	Gln	Ala	Phe	Leu	Asn	Arg	Phe	Arg	Asp	
785					790					795					800	

Leu Gly Arg Leu Arg Gly Leu Asp Thr Ser Ser Val Ala Pro Ser Ala	805	810	815
Pro Gly Cys His His Gly Pro Ile Pro Pro Gly Arg Arg His His Cys	820	825	830
Arg Ser Lys Ala Lys Arg Ser Arg His His His His Gln Thr Pro Arg	835	840	845
Pro Glu Thr Pro Cys Tyr Val Ser His Pro Ser Pro Val Pro Ser Ser	850	855	860
Gly Pro Trp Pro Pro Pro Pro Ala Thr Thr Pro Phe Pro Ala Met Val	865	870	875
Gln Pro Tyr Pro Leu Pro Val Phe Ser Pro Arg Gly Gly Pro Gln Pro	885	890	895
Leu Pro Pro Ala Pro Thr Ser Val Ser Pro Ala Thr Phe Pro Ser Pro	900	905	910
Leu Val Thr Pro Met Val Ala Leu Val Leu Pro Asn Tyr Leu Phe Pro	915	920	925
Thr Pro Pro Ser Tyr Pro Tyr Gly Val Ser Gln Ala Pro Val Glu Gly	930	935	940
Pro Pro Thr Pro Ala Ser His Ser Pro Ser Pro Ser Leu Pro Pro Pro	945	950	955
Pro Leu Ser Pro Pro His Arg Pro Asp Ser Pro Leu Phe Asn Ser Arg	965	970	975
Cys Ser Ser Pro Leu Gln Leu Asn Leu Leu Gln Leu Glu Glu Ser Pro	980	985	990
Arg Thr Glu Gly Gly Ala Ala Ala Gly Gly Pro Gly Ser Ser Ala Gly	995	1000	1005

Pro Leu Pro Pro Ser Glu Glu Thr Ala Glu Pro Glu Ala Arg Leu Val  
 1010 1015 1020  
 Glu Val Thr Glu Ser Ser Asn Gln Asp Ala Leu Ser Gly Ser Ser Asp  
 1025 1030 1035 1040  
 Leu Leu Glu Leu Leu Leu Gln Glu Asp Ser Arg Ser Gly Thr Gly Ser  
 1045 1050 1055  
 Ala Ala Ser Gly Ser Leu Gly Ser Gly Leu Gly Ser Gly Ser Gly Ser  
 1060 1065 1070  
 Gly Ser His Glu Gly Gly Ser Thr Ser Ala Ser Ile Thr Arg Ser Ser  
 1075 1080 1085  
 Gln Ser Ser His Thr Ser Lys Tyr Phe Gly Ser Ile Asp Ser Ser Glu  
 1090 1095 1100  
 Ala Glu Ala Gly Ala Ala Arg Ala Arg Thr Glu Pro Gly Asp Gln Val  
 1105 1110 1115 1120  
 Ile Lys Cys Val Leu Gln Asp Pro Ile Trp Leu Leu Met Ala Asn Ala  
 1125 1130 1135  
 Asp Gln Arg Val Met Met Thr Tyr Gln Val Pro Ser Arg Asp Ala Ala  
 1140 1145 1150  
 Ser Val Leu Lys Gln Asp Arg Glu Arg Leu Arg Ala Met Gln Lys Gln  
 1155 1160 1165  
 Gln Pro Arg Phe Ser Glu Asp Gln Arg Arg Glu Leu Gly Ala Val His  
 1170 1175 1180  
 Ser Trp Val Arg Lys Gly Gln Leu Pro Arg Ala Leu Asp Val Met Ala  
 1185 1190 1195 1200  
 Cys Val Asp Cys Gly Ser Ser Val Gln Asp Pro Gly His Ser Asp Asp

1205	1210	1215
Pro Leu Phe Ser Glu Leu Asp Gly Leu Gly Leu Glu Pro Met Glu Glu		
1220	1225	1230
Gly Gly Gly Glu Gly Gly Gly Cys Gly Val Gly Gly Gly Gly Asp		
1235	1240	1245
Gly Gly Glu Glu Ala Gln Thr Gln Ile Gly Ala Lys Gly Ser Ser Ser		
1250	1255	1260
Gln Asp Ser Ala Met Glu Glu Glu Gln Gly Gly Gly Ser Ser Ser		
1265	1270	1275 1280
Pro Ala Leu Pro Ala Glu Glu Asn Ser Thr Ser		
1285	1290	

<210> 3  
 <211> 3873  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> CDS  
 <222> (1)..(3873)

<400> 3  
 atg agt ggc ccc cta gaa ggg gct gat ggg gga ggg gac ccc agg cct 48  
 Met Ser Gly Pro Leu Glu Gly Ala Asp Gly Gly Gly Asp Pro Arg Pro  
 1 5 10 15  
 ggg gaa tca ttt tgt cct ggg ggc gtc cca tcc cct ggg ccc cca cag 96  
 Gly Glu Ser Phe Cys Pro Gly Gly Val Pro Ser Pro Gly Pro Pro Gln  
 20 25 30  
 cac cgg cct tgc cca ggc ccc agc ctg gcc gat gac acc gat gcc aac 144  
 His Arg Pro Cys Pro Gly Pro Ser Leu Ala Asp Asp Thr Asp Ala Asn  
 35 40 45

agc aat ggt tca agt ggc aat gag tcc aac ggg cat gag tct aga ggc	192
Ser Asn Gly Ser Ser Gly Asn Glu Ser Asn Gly His Glu Ser Arg Gly	
50 55 60	
 gca tct cag cgg agc tca cac agc tcc tcc tca ggc aac ggc aag gac	240
Ala Ser Gln Arg Ser Ser His Ser Ser Ser Ser Gly Asn Gly Lys Asp	
65 70 75 80	
 tca gcc ctg ctg gag acc act gag agc agc aag agc aca aac tct cag	288
Ser Ala Leu Leu Glu Thr Thr Glu Ser Ser Lys Ser Thr Asn Ser Gln	
85 90 95	
 agc cca tcc cca ccc agc agt tcc att gcc tac agc ctc ctg agt gcc	336
Ser Pro Ser Pro Pro Ser Ser Ser Ile Ala Tyr Ser Leu Leu Ser Ala	
100 105 110	
 agc tca gag cag gac aac ccg tcc acc agt ggc tgc agc agt gaa cag	384
Ser Ser Glu Gln Asp Asn Pro Ser Thr Ser Gly Cys Ser Ser Glu Gln	
115 120 125	
 tca gcc cgg gca agg act cag aag gaa ctc atg aca gca ctt cga gag	432
Ser Ala Arg Ala Arg Thr Gln Lys Glu Leu Met Thr Ala Leu Arg Glu	
130 135 140	
 ctc aag ctt cga ctg ccg cca gag cgc cgg ggc aag ggc cgc tct ggg	480
Leu Lys Leu Arg Leu Pro Pro Glu Arg Arg Gly Lys Gly Arg Ser Gly	
145 150 155 160	
 acc ctg gcc acg ctg cag tac gca ctg gcc tgt gtc aag cag gtg cag	528
Thr Leu Ala Thr Leu Gln Tyr Ala Leu Ala Cys Val Lys Gln Val Gln	
165 170 175	
 gcc aac cag gaa tac tac cag cag tgg agc ctg gag gag ggc gag cct	576
Ala Asn Gln Glu Tyr Tyr Gln Gln Trp Ser Leu Glu Glu Gly Glu Pro	
180 185 190	
 tgc tcc atg gac atg tcc acc tat acc ctg gag gag ctg gag cac atc	624

Cys	Ser	Met	Asp	Met	Ser	Thr	Tyr	Thr	Leu	Glu	Glu	Leu	Glu	His	Ile	
		195					200					205				
acg	tct	gag	tac	aca	ctt	cag	aac	cag	gat	acc	ttc	tca	gtg	gct	gtc	672
Thr	Ser	Glu	Tyr	Thr	Leu	Gln	Asn	Gln	Asp	Thr	Phe	Ser	Val	Ala	Val	
	210					215					220					
tcc	ttc	ctg	acg	ggc	cga	atc	gtc	tac	att	tcg	gag	cag	gca	gcc	gtc	720
Ser	Phe	Leu	Thr	Gly	Arg	Ile	Val	Tyr	Ile	Ser	Glu	Gln	Ala	Ala	Val	
225					230					235				240		
ctg	ctg	cgt	tgc	aag	cgg	gac	gtg	ttc	cgg	ggt	acc	cgc	ttc	tct	gag	768
Leu	Leu	Arg	Cys	Lys	Arg	Asp	Val	Phe	Arg	Gly	Thr	Arg	Phe	Ser	Glu	
			245					250					255			
ctc	ctg	gct	ccc	cag	gat	gtg	gga	gtc	ttc	tat	ggt	tcc	act	gct	cca	816
Leu	Leu	Ala	Pro	Gln	Asp	Val	Gly	Val	Phe	Tyr	Gly	Ser	Thr	Ala	Pro	
		260					265					270				
tct	cgc	ctg	ccc	acc	tgg	ggc	aca	ggg	gcc	tca	gca	ggt	tca	ggc	ctc	864
Ser	Arg	Leu	Pro	Thr	Trp	Gly	Thr	Gly	Ala	Ser	Ala	Gly	Ser	Gly	Leu	
	275					280					285					
agg	gac	ttt	acc	cag	gag	aag	tcc	gtc	ttc	tgc	cgt	atc	aga	gga	ggt	912
Arg	Asp	Phe	Thr	Gln	Glu	Lys	Ser	Val	Phe	Cys	Arg	Ile	Arg	Gly	Gly	
	290					295				300						
cct	gac	cgg	gat	cca	ggg	cct	cgg	tac	cag	cca	ttc	cgc	cta	acc	ccg	960
Pro	Asp	Arg	Asp	Pro	Gly	Pro	Arg	Tyr	Gln	Pro	Phe	Arg	Leu	Thr	Pro	
305				310						315				320		
tat	gtg	acc	aag	atc	cgg	gtc	tca	gat	ggg	gcc	cct	gca	cag	ccg	tgc	1008
Tyr	Val	Thr	Lys	Ile	Arg	Val	Ser	Asp	Gly	Ala	Pro	Ala	Gln	Pro	Cys	
			325						330				335			
tgc	ctg	ctg	att	gca	gag	cgc	atc	cat	tcg	ggt	tac	gaa	gct	ccc	cgg	1056
Cys	Leu	Leu	Ile	Ala	Glu	Arg	Ile	His	Ser	Gly	Tyr	Glu	Ala	Pro	Arg	
	340							345				350				

ata ccc cct gac aag agg att ttc act acg cgg cac aca ccc agc tgc 1104  
 Ile Pro Pro Asp Lys Arg Ile Phe Thr Thr Arg His Thr Pro Ser Cys  
 355 360 365

ctc ttc cag gat gtg gat gaa agg gct gcc ccc ctg ctg ggc tac ctg 1152  
 Leu Phe Gln Asp Val Asp Glu Arg Ala Ala Pro Leu Leu Gly Tyr Leu  
 370 375 380

ccc cag gac ctc ctg ggg gcc cca gtg ctc ctg ttc ctg cat cct gag 1200  
 Pro Gln Asp Leu Leu Gly Ala Pro Val Leu Leu Phe Leu His Pro Glu  
 385 390 395 400

gac cga ccc ctc atg ctg gct atc cac aag aag att ctg cag ttg gcg 1248  
 Asp Arg Pro Leu Met Leu Ala Ile His Lys Lys Ile Leu Gln Leu Ala  
 405 410 415

ggc cag ccc ttt gac cac tcc cct atc cgc ttc tgt gcc cgc aac ggg 1296  
 Gly Gln Pro Phe Asp His Ser Pro Ile Arg Phe Cys Ala Arg Asn Gly  
 420 425 430

gag tat gtc acc atg gac acc agc tgg gct ggc ttt gtg cac ccc tgg 1344  
 Glu Tyr Val Thr Met Asp Thr Ser Trp Ala Gly Phe Val His Pro Trp  
 435 440 445

agc cgc aag gta gcc ttc gtg ttg ggc cgc cac aaa gta cgc acg gcc 1392  
 Ser Arg Lys Val Ala Phe Val Leu Gly Arg His Lys Val Arg Thr Ala  
 450 455 460

ccc ctg aat gag gac gtg ttc act ccc ccg gcc ccc agc cca gct ccc 1440  
 Pro Leu Asn Glu Asp Val Phe Thr Pro Pro Ala Pro Ser Pro Ala Pro  
 465 470 475 480

tcc ctg gac act gat atc cag gag ctg tca gag cag atc cac cgg ctg 1488  
 Ser Leu Asp Thr Asp Ile Gln Glu Leu Ser Glu Gln Ile His Arg Leu  
 485 490 495

ctg ctg cag ccc gtc cac agc ccc agc ccc acg gga ctc tgt gga gtc 1536



Leu	Leu	Gln	Pro	Val	His	Ser	Pro	Ser	Pro	Thr	Gly	Leu	Cys	Gly	Val		
			500					505					510				
ggc	gcc	gtg	aca	tcc	cca	ggc	cct	ctc	cac	agc	cct	ggg	tcc	tcc	agt	1584	
Gly	Ala	Val	Thr	Ser	Pro	Gly	Pro	Leu	His	Ser	Pro	Gly	Ser	Ser	Ser		
			515				520					525					
gat	agc	aac	ggg	ggt	gat	gca	gag	ggg	cct	ggg	cct	cct	gcg	cca	gtg	1632	
Asp	Ser	Asn	Gly	Gly	Asp	Ala	Glu	Gly	Pro	Gly	Pro	Pro	Ala	Pro	Val		
			530				535				540						
act	ttc	cag	cag	atc	tgt	aag	gat	gtg	cat	ctg	gtg	aag	cac	cag	ggc	1680	
Thr	Phe	Gln	Gln	Ile	Cys	Lys	Asp	Val	His	Leu	Val	Lys	His	Gln	Gly		
						550				555					560		
cag	cag	ctt	ttt	att	gag	tct	cgg	gcc	cgg	cct	cag	tcc	cgg	ccc	cgc	1728	
Gln	Gln	Leu	Phe	Ile	Glu	Ser	Arg	Ala	Arg	Pro	Gln	Ser	Arg	Pro	Arg		
						565				570					575		
ctc	cct	gct	aca	ggc	acg	ttc	aag	gcc	aag	gcc	ctt	ccc	tgc	caa	tcc	1776	
Leu	Pro	Ala	Thr	Gly	Thr	Phe	Lys	Ala	Lys	Ala	Leu	Pro	Cys	Gln	Ser		
						580				585					590		
cca	gac	cca	gag	ctg	gag	gcg	ggt	tct	gct	ccc	gtc	cag	gcc	cca	cta	1824	
Pro	Asp	Pro	Glu	Leu	Glu	Ala	Gly	Ser	Ala	Pro	Val	Gln	Ala	Pro	Leu		
						595				600					605		
gcc	ttg	gtc	cct	gag	gag	gcc	gag	agg	aaa	gaa	gcc	tcc	agc	tgc	tcc	1872	
Ala	Leu	Val	Pro	Glu	Glu	Ala	Glu	Arg	Lys	Glu	Ala	Ser	Ser	Cys	Ser		
						610									620		
tac	cag	cag	atc	aac	tgc	ctg	gac	agc	atc	ctc	agg	tac	ctg	gag	agc	1920	
Tyr	Gln	Gln	Ile	Asn	Cys	Leu	Asp	Ser	Ile	Leu	Arg	Tyr	Leu	Glu	Ser		
						625					635				640		
tgc	aac	ctc	ccc	agc	acc	act	aag	cgt	aaa	tgt	gcc	tcc	tcc	tcc	tcc	1968	
Cys	Asn	Leu	Pro	Ser	Thr	Thr	Lys	Arg	Lys	Cys	Ala	Ser	Ser	Ser	Ser		
							645				650				655		

tat acc acc tcc tca gcc tct gac gac gac agg cag agg aca ggt cca 2016  
 Tyr Thr Thr Ser Ser Ala Ser Asp Asp Asp Arg Gln Arg Thr Gly Pro  
 660 665 670

gtc tct gtg ggg acc aag aaa gat ccg ccg tca gca gcg ctg tct ggg 2064  
 Val Ser Val Gly Thr Lys Lys Asp Pro Pro Ser Ala Ala Leu Ser Gly  
 675 680 685

gag ggg gcc acc cca cgg aag gag cca gtg gtg gga ggc acc ctg agc 2112  
 Glu Gly Ala Thr Pro Arg Lys Glu Pro Val Val Gly Gly Thr Leu Ser  
 690 695 700

ccg ctc gcc ctg gcc aat aag gcg gag agt gtg gtg tcc gtc acc agt 2160  
 Pro Leu Ala Leu Ala Asn Lys Ala Glu Ser Val Val Ser Val Thr Ser  
 705 710 715 720

cag tgt agc ttc agc tcc acc atc gtc cat gtg gga gac aag aag ccc 2208  
 Gln Cys Ser Phe Ser Ser Thr Ile Val His Val Gly Asp Lys Lys Pro  
 725 730 735

ccg gag tcg gac atc atc atg atg gag gac ctg cct ggc cta gcc cca 2256  
 Pro Glu Ser Asp Ile Ile Met Met Glu Asp Leu Pro Gly Leu Ala Pro  
 740 745 750

ggc cca gcc ccc agc cca gcc ccc agc ccc aca gta gcc cct gac cca 2304  
 Gly Pro Ala Pro Ser Pro Ala Pro Ser Pro Thr Val Ala Pro Asp Pro  
 755 760 765

gcc cca gac gcc tac cgt cca gtg ggg ctg acc aag gcc gtg ctg tcc 2352  
 Ala Pro Asp Ala Tyr Arg Pro Val Gly Leu Thr Lys Ala Val Leu Ser  
 770 775 780

ctg cac aca cag aag gaa gag caa gcc ttc ctc agc cgc ttc cga gac 2400  
 Leu His Thr Gln Lys Glu Glu Gln Ala Phe Leu Ser Arg Phe Arg Asp  
 785 790 795 800

ctg ggc agg ctg cgt gga ctc gac agc tct tcc aca gct ccc tca gcc 2448

005050 "ethc30500"

Leu Gly Arg	Leu Arg Gly	Leu Asp Ser	Ser Ser Ser	Thr Ala Pro	Ser Ala	
	805		810		815	
ctt ggc gag cga ggc tgc cac cac ggc ccc gca ccc cca agc cgc cga						2496
Leu Gly Glu Arg Gly Cys His His Gly Pro Ala Pro Pro Ser Arg Arg						
	820		825		830	
cac cac tgc cga tcc aaa gcc aag cgc tca cgc cac cac cag aac cct						2544
His His Cys Arg Ser Lys Ala Lys Arg Ser Arg His His Gln Asn Pro						
	835		840		845	
cgg gct gaa gcg ccc tgc tat gtc tca cac ccc tca ccc gtg cca ccc						2592
Arg Ala Glu Ala Pro Cys Tyr Val Ser His Pro Ser Pro Val Pro Pro						
	850		855		860	
tcc acc ccc tgg ccc acc cca cca gcc act acc ccc ttc cca gcg gtt						2640
Ser Thr Pro Trp Pro Thr Pro Pro Ala Thr Thr Pro Phe Pro Ala Val						
	865		870		875	880
gtc cag ccc tac cct ctc cca gtg ttc tct cct cga gga ggc ccc cag						2688
Val Gln Pro Tyr Pro Leu Pro Val Phe Ser Pro Arg Gly Gly Pro Gln						
		885		890		895
cct ctt ccc cct gct ccc aca tct gtg ccc cca gct gct ttc ccc gcc						2736
Pro Leu Pro Pro Ala Pro Thr Ser Val Pro Pro Ala Ala Phe Pro Ala						
	900		905		910	
cct ttg gtg acc cca atg gtg gcc ttg gtg ctc cct aac tat ctg ttc						2784
Pro Leu Val Thr Pro Met Val Ala Leu Val Leu Pro Asn Tyr Leu Phe						
	915		920		925	
cca acc cca tcc agc tat cct tat ggg gca ctc cag acc cct gct gaa						2832
Pro Thr Pro Ser Ser Tyr Pro Tyr Gly Ala Leu Gln Thr Pro Ala Glu						
	930		935		940	
ggg cct ccc act cct gcc tcg cac tcc cct tct cca tcc ttg ccc gcc						2880
Gly Pro Pro Thr Pro Ala Ser His Ser Pro Ser Pro Ser Leu Pro Ala						
	945		950		955	960

ctc	ccc	ccg	agt	cct	cct	cac	cgc	ccg	gac	tct	cca	ctg	ttc	aac	tcg	2928
Leu	Pro	Pro	Ser	Pro	Pro	His	Arg	Pro	Asp	Ser	Pro	Leu	Phe	Asn	Ser	
				965					970					975		
aga	tgc	agc	tct	cca	ctc	cag	ctc	aat	ctg	ctg	cag	ctg	gag	gag	ctc	2976
Arg	Cys	Ser	Ser	Pro	Leu	Gln	Leu	Asn	Leu	Leu	Gln	Leu	Glu	Glu	Leu	
			980					985					990			
ccc	cgt	gct	gag	ggg	gct	gct	gtt	gca	gga	ggc	cct	ggg	agc	agt	gcc	3024
Pro	Arg	Ala	Glu	Gly	Ala	Ala	Val	Ala	Gly	Gly	Pro	Gly	Ser	Ser	Ala	
			995				1000					1005				
ggg	ccc	cca	cct	ccc	agt	gcg	gag	gct	gct	gag	cca	gag	gcc	aga	ctg	3072
Gly	Pro	Pro	Pro	Pro	Ser	Ala	Glu	Ala	Ala	Glu	Pro	Glu	Ala	Arg	Leu	
	1010					1015				1020						
gcg	gag	gtc	act	gag	tcc	tcc	aat	cag	gac	gca	ctt	tcc	ggc	tcc	agt	3120
Ala	Glu	Val	Thr	Glu	Ser	Ser	Asn	Gln	Asp	Ala	Leu	Ser	Gly	Ser	Ser	
1025				1030					1035					1040		
gac	ctg	ctc	gaa	ctt	ctg	ctg	caa	gag	gac	tcg	cgc	tcc	ggc	aca	ggc	3168
Asp	Leu	Leu	Glu	Leu	Leu	Leu	Gln	Glu	Asp	Ser	Arg	Ser	Gly	Thr	Gly	
			1045					1050					1055			
tcc	gca	gcc	tcg	ggc	tcc	ttg	ggc	tct	ggc	ttg	ggc	tct	ggg	tct	ggt	3216
Ser	Ala	Ala	Ser	Gly	Ser	Leu	Gly	Ser	Gly	Leu	Gly	Ser	Gly	Ser	Gly	
			1060				1065					1070				
tca	ggc	tcc	cat	gaa	ggg	ggc	agc	acc	tca	gcc	agc	atc	act	cgc	agc	3264
Ser	Gly	Ser	His	Glu	Gly	Gly	Ser	Thr	Ser	Ala	Ser	Ile	Thr	Arg	Ser	
			1075				1080					1085				
agc	cag	agc	agc	cac	aca	agc	aaa	tac	ttt	ggc	agc	atc	gac	tct	tcc	3312
Ser	Gln	Ser	Ser	His	Thr	Ser	Lys	Tyr	Phe	Gly	Ser	Ile	Asp	Ser	Ser	
	1090					1095				1100						
gag	gct	gag	gct	ggg	gct	gct	cgg	ggc	ggg	gct	gag	cct	ggg	gac	cag	3360

Glu Ala Glu Ala Gly Ala Ala Arg Gly Gly Ala Glu Pro Gly Asp Gln  
 1105 1110 1115 1120  
 gtg att aag tac gtg ctc cag gat ccc att tgg ctg ctc atg gcc aat 3408  
 Val Ile Lys Tyr Val Leu Gln Asp Pro Ile Trp Leu Leu Met Ala Asn  
 1125 1130 1135  
 gct gac cag cgc gtc atg atg acc tac cag gtg ccc tcc agg gac atg 3456  
 Ala Asp Gln Arg Val Met Met Thr Tyr Gln Val Pro Ser Arg Asp Met  
 1140 1145 1150  
 acc tct gtg ctg aag cag gat cgg gag cgg ctc cga gcc atg cag aag 3504  
 Thr Ser Val Leu Lys Gln Asp Arg Glu Arg Leu Arg Ala Met Gln Lys  
 1155 1160 1165  
 cag cag cct cgg ttt tct gag gac cag cgg cgg gaa ctg ggt gct gtg 3552  
 Gln Gln Pro Arg Phe Ser Glu Asp Gln Arg Arg Glu Leu Gly Ala Val  
 1170 1175 1180  
 cac tcc tgg gtc cgg aag ggc caa ctg cct cgg gct ctt gat gtg atg 3600  
 His Ser Trp Val Arg Lys Gly Gln Leu Pro Arg Ala Leu Asp Val Met  
 1185 1190 1195 1200  
 gcc tgt gtg gac tgt ggg agc agc acc caa gat cct ggt cac cct gat 3648  
 Ala Cys Val Asp Cys Gly Ser Ser Thr Gln Asp Pro Gly His Pro Asp  
 1205 1210 1215  
 gac cca ctc ttc tca gag ctg gat gga ctg ggg ctg gag ccc atg gaa 3696  
 Asp Pro Leu Phe Ser Glu Leu Asp Gly Leu Gly Leu Glu Pro Met Glu  
 1220 1225 1230  
 gag ggt gga ggc gag cag ggc agc agc ggt ggc ggc agt ggt gag gga 3744  
 Glu Gly Gly Gly Glu Gln Gly Ser Ser Gly Gly Gly Ser Gly Glu Gly  
 1235 1240 1245  
 gag ggc tgc gag gag gcc caa ggc ggg gcc aag gct tca agc tct cag 3792  
 Glu Gly Cys Glu Glu Ala Gln Gly Gly Ala Lys Ala Ser Ser Ser Gln  
 1250 1255 1260

0050341 0050342  
 0050343 0050344  
 0050345 0050346  
 0050347 0050348  
 0050349 0050350  
 0050351 0050352  
 0050353 0050354  
 0050355 0050356  
 0050357 0050358  
 0050359 0050360  
 0050361 0050362  
 0050363 0050364  
 0050365 0050366  
 0050367 0050368  
 0050369 0050370  
 0050371 0050372  
 0050373 0050374  
 0050375 0050376  
 0050377 0050378  
 0050379 0050380  
 0050381 0050382  
 0050383 0050384  
 0050385 0050386  
 0050387 0050388  
 0050389 0050390  
 0050391 0050392  
 0050393 0050394  
 0050395 0050396  
 0050397 0050398  
 0050399 0050400

gac ttg gct atg gag gag gag gaa gaa ggc agg agc tca tcc agt cca 3840  
 Asp Leu Ala Met Glu Glu Glu Glu Glu Gly Arg Ser Ser Ser Ser Pro  
 1265 1270 1275 1280

gcc tta cct aca gca gga aac tgc acc agc tag 3873  
 Ala Leu Pro Thr Ala Gly Asn Cys Thr Ser  
 1285 1290

<210> 4

<211> 3876

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1)..(3876)

<400> 4

atg agt ggt ccc cta gaa ggg gcc gat ggg gga gga gac ccc agg ccc 48  
 Met Ser Gly Pro Leu Glu Gly Ala Asp Gly Gly Gly Asp Pro Arg Pro  
 1 5 10 15

gga gaa cct ttt tgt cct gga gga gtc cca tcc cct ggg gcc ccg cag 96  
 Gly Glu Pro Phe Cys Pro Gly Gly Val Pro Ser Pro Gly Ala Pro Gln  
 20 25 30

cac cgg cct tgt cca ggc ccc agc ctg gct gat gac act gat gca aac 144  
 His Arg Pro Cys Pro Gly Pro Ser Leu Ala Asp Asp Thr Asp Ala Asn  
 35 40 45

agc aat ggc tca agt ggc aat gag tcc aac gga ccc gag tcc agg ggc 192  
 Ser Asn Gly Ser Ser Gly Asn Glu Ser Asn Gly Pro Glu Ser Arg Gly  
 50 55 60

gca tct cag cgg agt tct cat agt tcc tct tct ggc aat ggc aag gac 240  
 Ala Ser Gln Arg Ser Ser His Ser Ser Ser Ser Gly Asn Gly Lys Asp  
 65 70 75 80

3876 "24630530"

tcc ttc ctg aca ggc cgg att gtc tat att tcg gag cag gca ggt gtc 720

Ser Phe Leu Thr Gly Arg Ile Val Tyr Ile Ser Glu Gln Ala Gly Val  
 225 230 235 240

ctg ctg cgt tgc aaa cgg gat gtg ttt cgg ggt gcc cgc ttc tca gag 768  
 Leu Leu Arg Cys Lys Arg Asp Val Phe Arg Gly Ala Arg Phe Ser Glu  
 245 250 255

ctc ctg gct ccc cag gat gtg ggt gtc ttc tat ggc tct act aca cca 816  
 Leu Leu Ala Pro Gln Asp Val Gly Val Phe Tyr Gly Ser Thr Thr Pro  
 260 265 270

tct cga ctg ccc acc tgg ggc act ggc acc tct gca ggt tca ggt ctc 864  
 Ser Arg Leu Pro Thr Trp Gly Thr Gly Thr Ser Ala Gly Ser Gly Leu  
 275 280 285

aag gac ttc acc cag gaa aag tct gtc ttc tgc cga atc aga gga ggt 912  
 Lys Asp Phe Thr Gln Glu Lys Ser Val Phe Cys Arg Ile Arg Gly Gly  
 290 295 300

cct gac cgg gat cca ggg cct cgg tac cag cca ttc cgc cta acc cca 960  
 Pro Asp Arg Asp Pro Gly Pro Arg Tyr Gln Pro Phe Arg Leu Thr Pro  
 305 310 315 320

tat gtg acc aag att cgg gtc tca gat gga gcc cct gca cag ccg tgc 1008  
 Tyr Val Thr Lys Ile Arg Val Ser Asp Gly Ala Pro Ala Gln Pro Cys  
 325 330 335

tgc cta ctc att gcc gag cgc atc cac tct ggt tat gaa gct ccc cgg 1056  
 Cys Leu Leu Ile Ala Glu Arg Ile His Ser Gly Tyr Glu Ala Pro Arg  
 340 345 350

atc cct cct gac aag agg atc ttc acc acc cga cac aca cca agc tgc 1104  
 Ile Pro Pro Asp Lys Arg Ile Phe Thr Thr Arg His Thr Pro Ser Cys  
 355 360 365

ctc ttc cag gat gta gat gaa agg gct gcc cca ctg ctg ggt tac ctt 1152  
 Leu Phe Gln Asp Val Asp Glu Arg Ala Ala Pro Leu Leu Gly Tyr Leu  
 370 375 380



ccc cag gat ctc ctg ggg gct cca gta ctt ctc ttt cta cat cct gag 1200  
 Pro Gln Asp Leu Leu Gly Ala Pro Val Leu Leu Phe Leu His Pro Glu  
 385 390 395 400

gac cga ccc ctc atg ctg gcc att cat aag aag ata ctg cag ctg gca 1248  
 Asp Arg Pro Leu Met Leu Ala Ile His Lys Lys Ile Leu Gln Leu Ala  
 405 410 415

ggc cag ccc ttt gac cat tcc cct att cgc ttc tgt gct cgg aac ggg 1296  
 Gly Gln Pro Phe Asp His Ser Pro Ile Arg Phe Cys Ala Arg Asn Gly  
 420 425 430

gaa tat gtc acc atg gac acc agc tgg gcc ggt ttt gtg cac ccc tgg 1344  
 Glu Tyr Val Thr Met Asp Thr Ser Trp Ala Gly Phe Val His Pro Trp  
 435 440 445

agc cgc aag gtg gct ttc gtg ttg ggt cgc cat aaa gtg cgc acg gca 1392  
 Ser Arg Lys Val Ala Phe Val Leu Gly Arg His Lys Val Arg Thr Ala  
 450 455 460

ccc ctg aat gag gac gtc ttc act ccc cca gcc ccc agc cca gct cgg 1440  
 Pro Leu Asn Glu Asp Val Phe Thr Pro Pro Ala Pro Ser Pro Ala Pro  
 465 470 475 480

tcc ctg gac tct gat atc cag gag ctc tca gag cag atc cat cga ttg 1488  
 Ser Leu Asp Ser Asp Ile Gln Glu Leu Ser Glu Gln Ile His Arg Leu  
 485 490 495

ctg ctg cag cct gtg cac agc tcc agc ccc acg ggg ctc tgt gga gtt 1536  
 Leu Leu Gln Pro Val His Ser Ser Ser Pro Thr Gly Leu Cys Gly Val  
 500 505 510

ggc cct ctg atg tcc cct ggt cct cta cac agc cct ggc tcc tcc agt 1584  
 Gly Pro Leu Met Ser Pro Gly Pro Leu His Ser Pro Gly Ser Ser Ser  
 515 520 525

gat agc aat ggg ggg gac gct gag ggg cct ggg cct cct gct cca gtg 1632

Asp	Ser	Asn	Gly	Gly	Asp	Ala	Glu	Gly	Pro	Gly	Pro	Pro	Ala	Pro	Val		
530						535					540						
act	ttc	cag	cag	atc	tgt	aag	gat	gtg	cat	ctg	gta	aag	cac	cag	gga	1680	
Thr	Phe	Gln	Gln	Ile	Cys	Lys	Asp	Val	His	Leu	Val	Lys	His	Gln	Gly		
545					550					555					560		
caa	cag	ctc	ttc	att	gaa	tct	cgg	gcc	aag	ccc	cca	ccc	cgg	ccc	cgc	1728	
Gln	Gln	Leu	Phe	Ile	Glu	Ser	Arg	Ala	Lys	Pro	Pro	Pro	Arg	Pro	Arg		
				565					570					575			
ctc	ctt	gct	aca	ggt	aca	ttc	aaa	gcc	aaa	gtc	ctt	ccc	tgc	cag	tcc	1776	
Leu	Leu	Ala	Thr	Gly	Thr	Phe	Lys	Ala	Lys	Val	Leu	Pro	Cys	Gln	Ser		
			580					585					590				
cca	aac	ccc	gaa	ctg	gag	gtg	gcc	cca	gtt	cct	gac	caa	gcc	tcg	tta	1824	
Pro	Asn	Pro	Glu	Leu	Glu	Val	Ala	Pro	Val	Pro	Asp	Gln	Ala	Ser	Leu		
		595					600					605					
gcc	ttg	gcc	cct	gag	gag	cca	gag	agg	aaa	gaa	acc	tct	ggc	tgt	tcc	1872	
Ala	Leu	Ala	Pro	Glu	Glu	Pro	Glu	Arg	Lys	Glu	Thr	Ser	Gly	Cys	Ser		
	610					615					620						
tac	cag	cag	atc	aac	tgc	ctg	gac	agc	atc	ctc	agg	tat	ttg	gag	agc	1920	
Tyr	Gln	Gln	Ile	Asn	Cys	Leu	Asp	Ser	Ile	Leu	Arg	Tyr	Leu	Glu	Ser		
625				630						635					640		
tgc	aac	att	ccc	agt	aca	acc	aag	cgt	aaa	tgt	gcc	tcc	tcc	tcc	tcc	1968	
Cys	Asn	Ile	Pro	Ser	Thr	Thr	Lys	Arg	Lys	Cys	Ala	Ser	Ser	Ser	Ser		
				645					650					655			
tac	act	gcc	tct	tca	gcc	tct	gat	gat	gac	aag	cag	agg	gca	ggt	cca	2016	
Tyr	Thr	Ala	Ser	Ser	Ala	Ser	Asp	Asp	Asp	Lys	Gln	Arg	Ala	Gly	Pro		
			660					665					670				
gtt	cct	gtg	ggg	gcc	aag	aaa	gat	cgg	tcg	tca	gca	atg	ctg	tct	ggg	2064	
Val	Pro	Val	Gly	Ala	Lys	Lys	Asp	Pro	Ser	Ser	Ala	Met	Leu	Ser	Gly		
			675				680					685					

gag ggg gca act cct cgg aag gag cca gtg gtg gga ggc acc ctg agc 2112  
 Glu Gly Ala Thr Pro Arg Lys Glu Pro Val Val Gly Gly Thr Leu Ser  
 690 695 700

ccg ctc gcc ctg gcc aat aag gca gag agc gtg gtg tcc gtc acc agt 2160  
 Pro Leu Ala Leu Ala Asn Lys Ala Glu Ser Val Val Ser Val Thr Ser  
 705 710 715 720

cag tgt agc ttc agc tcc acc atc gtc cat gtg gga gac aag aag ccc 2208  
 Gln Cys Ser Phe Ser Ser Thr Ile Val His Val Gly Asp Lys Lys Pro  
 725 730 735

ccg gag tcg gac atc atc atg atg gaa gac ctg cct ggc ctg gcc cct 2256  
 Pro Glu Ser Asp Ile Ile Met Met Glu Asp Leu Pro Gly Leu Ala Pro  
 740 745 750

ggc cca gcc ccc agt ccg gcc ccc agc ccc aca gta gcc cct gac cca 2304  
 Gly Pro Ala Pro Ser Pro Ala Pro Ser Pro Thr Val Ala Pro Asp Pro  
 755 760 765

acc cca gat gct tat cgc cca gtg ggt ctg acc aag gcc gtg ctg tcc 2352  
 Thr Pro Asp Ala Tyr Arg Pro Val Gly Leu Thr Lys Ala Val Leu Ser  
 770 775 780

ctg cac aca cag aag gaa gag caa gcc ttc ctc aac cgc ttc aga gat 2400  
 Leu His Thr Gln Lys Glu Glu Gln Ala Phe Leu Asn Arg Phe Arg Asp  
 785 790 795 800

ctt ggc agg ctt cgt gga ctt gac acc tct tct gtg gcc ccc tca gcc 2448  
 Leu Gly Arg Leu Arg Gly Leu Asp Thr Ser Ser Val Ala Pro Ser Ala  
 805 810 815

cct ggc tgc cac cat ggc ccc att ccc cct ggt cgc cga cac cac tgc 2496  
 Pro Gly Cys His His Gly Pro Ile Pro Pro Gly Arg Arg His His Cys  
 820 825 830

cga tct aaa gca aag cgt tcc cgc cac cac cac cac cag acc ccc cgg 2544

2112  
 2160  
 2208  
 2256  
 2304  
 2352  
 2400  
 2448  
 2496  
 2544

Arg	Ser	Lys	Ala	Lys	Arg	Ser	Arg	His	His	His	His	Gln	Thr	Pro	Arg	
835				840				845								
ccc	gaa	act	ccc	tgc	tat	gtc	tcc	cat	cct	tca	cct	gtg	ccc	tct	tct	2592
Pro	Glu	Thr	Pro	Cys	Tyr	Val	Ser	His	Pro	Ser	Pro	Val	Pro	Ser	Ser	
850				855				860								
gga	ccc	tgg	cca	ccc	cca	cca	gcc	acg	acc	ccc	ttc	cca	gca	atg	gtc	2640
Gly	Pro	Trp	Pro	Pro	Pro	Pro	Ala	Thr	Thr	Pro	Phe	Pro	Ala	Met	Val	
865				870				875				880				
cag	ccc	tac	cca	ctc	cca	gta	ttc	tcc	cct	cga	gga	gga	ccc	cag	ccc	2688
Gln	Pro	Tyr	Pro	Leu	Pro	Val	Phe	Ser	Pro	Arg	Gly	Gly	Pro	Gln	Pro	
885				890				895								
ctt	ccc	cct	gcc	cct	aca	tct	gtg	tcc	cct	gct	acc	ttc	cct	tct	ccc	2736
Leu	Pro	Pro	Ala	Pro	Thr	Ser	Val	Ser	Pro	Ala	Thr	Phe	Pro	Ser	Pro	
900				905				910								
tta	gtg	acc	cca	atg	gtg	gcc	ttg	gtg	ctc	cct	aac	tat	cta	ttc	cct	2784
Leu	Val	Thr	Pro	Met	Val	Ala	Leu	Val	Leu	Pro	Asn	Tyr	Leu	Phe	Pro	
915				920				925								
acc	cca	cct	agt	tat	cca	tat	ggg	gtg	tcc	cag	gcc	cct	gtt	gag	ggg	2832
Thr	Pro	Pro	Ser	Tyr	Pro	Tyr	Gly	Val	Ser	Gln	Ala	Pro	Val	Glu	Gly	
930				935				940								
cca	ccc	acg	cct	gct	tcc	cac	tcg	ccc	tct	cca	tcc	ctg	ccc	cca	cca	2880
Pro	Pro	Thr	Pro	Ala	Ser	His	Ser	Pro	Ser	Pro	Ser	Leu	Pro	Pro	Pro	
945				950				955				960				
cct	ctc	agc	ccc	ccc	cac	cgc	cca	gac	tcc	cca	ctg	ttc	aac	tcg	aga	2928
Pro	Leu	Ser	Pro	Pro	His	Arg	Pro	Asp	Ser	Pro	Leu	Phe	Asn	Ser	Arg	
965				970				975								
tgc	agc	tcc	cca	ctc	cag	ctc	aat	ctg	ctg	cag	ctt	gag	gag	tcc	ccc	2976
Cys	Ser	Ser	Pro	Leu	Gln	Leu	Asn	Leu	Leu	Gln	Leu	Glu	Glu	Ser	Pro	
980				985				990								

cgc acg gag ggg ggc gct gct gca gga ggc cca gga agc agt gct ggg	3024
Arg Thr Glu Gly Gly Ala Ala Ala Gly Gly Pro Gly Ser Ser Ala Gly	
995 1000 1005	
ccc ctg cct ccc agt gag gag act gct gag cca gag gcc aga ttg gtg	3072
Pro Leu Pro Pro Ser Glu Glu Thr Ala Glu Pro Glu Ala Arg Leu Val	
1010 1015 1020	
gag gtt act gag tcg tcc aat cag gat gca ctt tca ggc tcc agc gac	3120
Glu Val Thr Glu Ser Ser Asn Gln Asp Ala Leu Ser Gly Ser Ser Asp	
1025 1030 1035 1040	
ctg ctg gag cta ctg ctc caa gaa gac tct cgc tcg ggc aca ggc tcc	3168
Leu Leu Glu Leu Leu Leu Gln Glu Asp Ser Arg Ser Gly Thr Gly Ser	
1045 1050 1055	
gca gcc tca ggc tcc ctg ggc tct ggc ctg ggc tct ggg tct ggt tca	3216
Ala Ala Ser Gly Ser Leu Gly Ser Gly Leu Gly Ser Gly Ser Gly Ser	
1060 1065 1070	
gga tcc cac gaa ggg gga agc acc tca gcc agc atc acc cgc agc agt	3264
Gly Ser His Glu Gly Gly Ser Thr Ser Ala Ser Ile Thr Arg Ser Ser	
1075 1080 1085	
cag agc agc cat aca agc aag tac ttt ggc agc atc gac tct tcc gag	3312
Gln Ser Ser His Thr Ser Lys Tyr Phe Gly Ser Ile Asp Ser Ser Glu	
1090 1095 1100	
gct gaa gct ggg gct gct cgg gcc agg act gag cct ggg gac cag gtc	3360
Ala Glu Ala Gly Ala Ala Arg Ala Arg Thr Glu Pro Gly Asp Gln Val	
1105 1110 1115 1120	
att aag tgt gtg ctc cag gac ccc atc tgg ctg ctc atg gcc aat gcc	3408
Ile Lys Cys Val Leu Gln Asp Pro Ile Trp Leu Leu Met Ala Asn Ala	
1125 1130 1135	
gac cag cgt gtc atg atg aca tac cag gtg cgg tcc agg gat gca gcc	3456

Asp Gln Arg Val Met Met Thr Tyr Gln Val Pro Ser Arg Asp Ala Ala  
 1140 1145 1150

tct gtg ctg aag caa gac cgg gag agg ctc cgg gcc atg cag aaa cag 3504  
 Ser Val Leu Lys Gln Asp Arg Glu Arg Leu Arg Ala Met Gln Lys Gln  
 1155 1160 1165

cag cca cgg ttc tca gag gac cag agg cgg gaa ctg ggt gct gtg cac 3552  
 Gln Pro Arg Phe Ser Glu Asp Gln Arg Arg Glu Leu Gly Ala Val His  
 1170 1175 1180

tcc tgg gtc cgg aag ggc cag ctg cct cgg gcc ctt gat gtg atg gcg 3600  
 Ser Trp Val Arg Lys Gly Gln Leu Pro Arg Ala Leu Asp Val Met Ala  
 1185 1190 1195 1200

tgt gtg gac tgt ggc agc agc gtt caa gat cct ggc cac tct gat gac 3648  
 Cys Val Asp Cys Gly Ser Ser Val Gln Asp Pro Gly His Ser Asp Asp  
 1205 1210 1215

ccg ctc ttc tca gaa ctg gat gga ttg ggg ctg gag ccc atg gaa gag 3696  
 Pro Leu Phe Ser Glu Leu Asp Gly Leu Gly Leu Glu Pro Met Glu Glu  
 1220 1225 1230

ggt gga ggc gag ggt ggt ggg tgt ggt gtt ggc ggc ggt ggg ggt gat 3744  
 Gly Gly Gly Glu Gly Gly Gly Cys Gly Val Gly Gly Gly Gly Gly Asp  
 1235 1240 1245

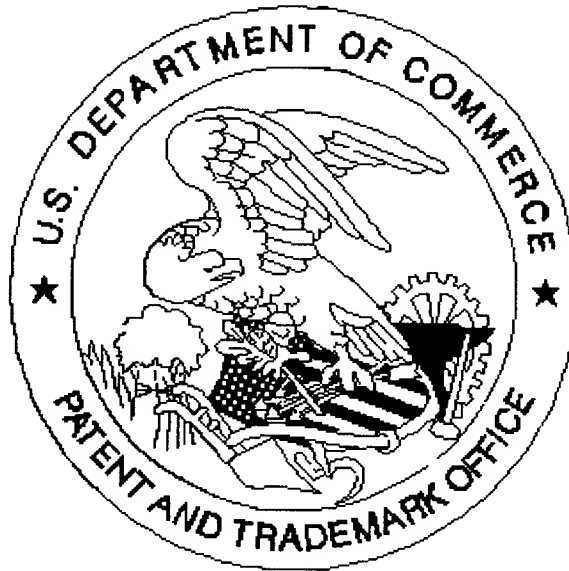
ggt ggt gag gag gcc cag acc caa att ggg gct aag ggt tca agc tct 3792  
 Gly Gly Glu Glu Ala Gln Thr Gln Ile Gly Ala Lys Gly Ser Ser Ser  
 1250 1255 1260

cag gac tct gcc atg gag gaa gaa gag caa ggt ggg ggc tca tcc agc 3840  
 Gln Asp Ser Ala Met Glu Glu Glu Glu Gln Gly Gly Gly Ser Ser Ser  
 1265 1270 1275 1280

cca gct tta cct gca gaa gaa aac agc acc agc tag 3876  
 Pro Ala Leu Pro Ala Glu Glu Asn Ser Thr Ser  
 1285 1290

005050 "ethers" 005050

United States Patent & Trademark Office  
Office of Initial Patent Examination -- Scanning Division



Application deficiencies found during scanning:

☐ Page(s) \_\_\_\_\_ of \_\_\_\_\_ were not present  
for scanning. (Document title)

☐ Page(s) \_\_\_\_\_ of \_\_\_\_\_ were not present  
for scanning. (Document title)

■ Scanned copy is best available. Drawing some fig are Dark

— Drawing, There are only 10 sheets of Drawings  
available for scanning, not 31 sheets